ROLE OF EXTRACELLULAR CALCIUM AND THE SODIUM-POTASSIUM PUMP IN ALTERATIONS OF VASCULAR REACTIVITY IN DOCA/NaCl HYPERTENSIVE RATS

By

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Abstract of Dissertation Presented to the Graduate
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ROLE OF EXTRACELLULAR CALCIUM AND THE SODIUM-POTASSIUM PUMP IN ALTERATIONS OF VASCULAR REACTIVITY IN DOCA/NaCl HYPERTENSIVE RATS

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The effect of alterations in extracellular calcium concentration and sodium-potassium pump activity on vascular reactivity in control and deoxycorticosterone acetate/1 percent sodium chloride (DOCA/NaCl) hypertensive rats was investigated.

An increase in the responsiveness of rings of femoral artery from DOCA/NaCl rats to potassium chloride (KCl) and norepinephrine (NE) was observed *in vitro*. Equilibration of tissues in a low calcium (0.25 mM) modified Krebs physiological solution resulted in a significant decrease in the reactivity of vascular smooth muscle (VSM) from DOCA/NaCl rats to both KCl and NE so that responses were similar to controls.

Sodium-potassium pump activity was estimated in femoral arteries of control and DOCA/NaCl hypertensive rats using three methods: potassium-relaxation of rings of femoral artery; rubidium-86 uptake; and calcium-relaxation of rings of femoral artery. Pump activity was significantly increased in VSM from the DOCA/NaCl rat as estimated by all three methods.

Femoral smooth muscle from control and DOCA/NaCl hypertensive rats was equilibrated in a Krebs physiological solution with varying concentrations of extracellular potassium to determine the role of the sodium-potassium pump in mediating vascular adrenergic responsiveness. The response of VSM from controls to NE was increased in a potassium-free (0 mM) Krebs solution when compared to responses in normal potassium. Equilibration in a 10 mM potassium Krebs solution or at 27° C had no effect on vascular reactivity. Rings of femoral artery from DOCA/NaCl hypertensive rats exhibited an enhanced response to NE at 27° C, while in a potassium-free Krebs solution the response was increased even further when compared to responses in normal potassium. Reactivity of VSM from the DOCA/NaCl rat in a high potassium (10 mM) Krebs physiological solution was decreased compared to responses in a normal potassium (5.9 mM) Krebs. The increased response in 0 mM potassium was greater in DOCA/NaCl rats than controls.

These results suggest that extracellular calcium and the sodium-potassium pump may play a significant role in

increasing peripheral vascular resistance in the DOCA/NaCl rat by increasing the sensitivity of the VSM to NE.

PART I
GENERAL INTRODUCTION

Hypertension or high blood pressure is defined as an abnormal condition characterized by an increase in total peripheral resistance and a persistent elevation of systolic and/or diastolic pressure(s). The World Health Organization has established that hypertension be designated as a systolic pressure greater than 160 mm Hg and/or a diastolic pressure greater than 95 mm Hg. Normotension is defined as systolic less than 140 mm Hg and diastolic less than 90 mm Hg. Pressures between these limits are considered borderline. Kaplan (1978), however, has suggested that these criteria should not be followed religiously, since they do not allow for factors such as age and sex. In addition, since blood pressure can vary markedly in a 24 hour period, it has become mandatory to repeat blood pressure measurements over several days to establish whether sustained hypertension is present.

Hypertension is present in approximately 15 to 20 percent of all adults worldwide and is considered the most common serious chronic health problem. It is estimated that 40 million Americans are afflicted with this disease, while another 25 million are considered borderline hypertensive. Stamler *et al*. (1976) screened 1 million Americans between 1974 and 1975 and confirmed the magnitude of this problem.

Almost 25 percent of the individuals had a diastolic pressure greater than 90 mm Hg and 12 percent greater than 95 mm Hg.

A number of distinct forms of hypertension exist throughout the population; however, they are generally placed into one of two major classifications.

Hypertension of unknown cause or etiology (i.e. essential or idiopathic hypertension) is generally referred to as primary hypertension. All other forms of hypertension, that is, those with a known cause, are classified as a secondary form of hypertension.

A number of renal diseases are known to cause hypertension, the most common being the result of renal artery stenosis. Reduction in renal blood flow by arterial occlusion or renal compression consistently results in an increase in blood pressure and more than likely involves the renin-angiotensin system. An elevation in blood pressure can occur as a result of the direct vasoconstrictor action of angiotensin or indirectly from the release of aldosterone causing an increase in blood volume.

Several diseases involving the adrenal gland are known to cause hypertension. Approximately 80 percent of the persons with Cushings syndrome exhibit an increase in blood pressure (O'Neal et al., 1970). Persons with both primary and secondary hyperaldosteronism have also been shown to have high blood pressure, but the incidence of hypertension is quite low. In experimental situations, the administration of deoxycorticosterone and salt to animals results in an increase in blood pressure (Selye et al., 1943).

Pheochromocytoma, another form of secondary hypertension, is a tumor of the chromaffin cells of the adrenal medulla as well as elsewhere in the body. These cells normally secrete norepinephrine and epinephrine in the body. However, in this condition there are periodic surges of catecholamine release which causes pallor, sweating, palpitations, and headache as well as the abrupt increase in blood pressure.

Persons with coarctation of the aorta generally present with an increased pressure in the upper extremities and a low pressure in the lower extremities. Although the renin-angiotensin system may not play a role in the cause of this form of secondary hypertension, it appears to be involved in the maintenance of this hypertensive state (Ribeiro and Krakoff, 1976).

Karlberg and Ottosson (1982) recently reported in a study that approximately 50 percent of the patients with acromegaly had hypertension. They observed that these subjects had alterations in the renin-angiotensin-aldosterone system with the growth hormone excess and suggested, as have others (Werning et al., 1970; Mantero et al., 1979), that the increase in blood pressure may be due to these alterations.

In recent years it has become increasingly apparent that a relationship exists between the use of oral contraceptives and the development of hypertension. Although the mechanism is not certain, it appears that these agents cause hypertension by increasing plasma renin and aldosterone as well as renin substrate (Low and Oparil, 1975).

The above list of secondary forms of hypertension comprises approximately 10 percent of the population with hypertension. The vast majority of hypertensives (approximately 90 percent) are classified as having essential hypertension (Manger and Page, 1982). As mentioned above, essential or primary hypertension is a condition in which there is a sustained elevation in systemic arterial blood pressure for which there is no discernable origin. This form of hypertension may be classified as "benign" or "malignant." Development of high blood pressure over many years is usually considered benign, while malignant connotates a rapid development over a short period of time.

A number of factors have been implicated in the increase in blood pressure. The prevalence of hypertension has been shown to increase with age in practically all populations (Borhani et al., 1968). In the Framingham studies it was also clearly indicated that mortality rates in hypertensives increased with age (Kannel and Sorlie, 1975). In the same study, though, it appeared that women tolerate hypertension better than men with mortality rates being lower for women. Blacks in the United States have substantially higher blood pressures than whites at any age and of either sex (Kaplan, 1978).

Heritable or familial influences on blood pressure are now well established in humans (Zinner *et al*., 1974; Laskarzewski *et al*., 1981). However, Miall (1978) has stated that even though genetic factors are important, evidence linking higher blood pressure to environmental factors remains strong.

A number of environmental factors have been implicated in the genesis of essential hypertension; however, a general consensus has been reached which considers the following three as the main influences on blood pressure: weight gain, salt intake, and psychosocial stress.

Experimental evidence has shown that the correlation between blood pressure and weight gain is highly significant (Johnson *et al*., 1975). Longitudinal studies showed an increase in blood pressure in individuals who are overweight and gain more weight, while reduction resulted in a decrease in blood pressure.

It is known that acute stress can cause a significant transient increase in blood pressure. However, the question remains unanswered whether repeated and prolonged stress can result in establishment of permanent hypertension. A number of studies have been done favoring this concept (Cobb and Rose, 1973; Jonsson and Hansson, 1977; Casell, 1974).

A growing interest in recent years has centered around the relationship of dietary sodium chloride to both clinical and experimental hypertension. Dietary sodium chloride has been implicated as a factor in the development of essential hypertension (Dahl and Love, 1957). Evidence in favor of this hypothesis is the observation that "primitive peoples" of the world who do not eat salt in their diet do not exhibit hypertension (Oliver _et al_., 1975). Furthermore, if these "primitive people" adapt to modern life-styles, and increase their sodium intake, their blood pressures rise (Maddocks, 1967).

Three recent studies (Parijs _et al_., 1977; Morgan _et al_., 1978; MacGregor _et al_., 1982) carried out on mild hypertensives demonstrated that a significant decrease in blood pressure occurred when the subjects were put on a modest sodium restricted diet.

It appears though that sodium restriction alone may not be maximally effective. Beretta-Piccoli _et al_. (1982) showed that plasma potassium concentration correlated inversely and significantly with blood pressure in hypertensive patients. In addition, Morgan (1982) showed that an increase in potassium in the diet reduces the blood pressure raising effects of sodium chloride. It is suggested, therefore, that a low sodium, high potassium diet would be more effective than sodium restriction alone.

In addition to the genetic and environmental factors listed above, several major hypotheses have been proposed as to the basic pathophysiological mechanism(s) involved in the genesis of hypertension.

One of these hypotheses involves an alteration in the function of the autonomic nervous system which is responsible for regulating blood pressure. Evidence consistent with this hypothesis comes from the observations of several investigators who showed that mean catecholamine levels are elevated in some hypertensive patients (Engelman *et al.*, 1970; Louis *et al.*, 1973; de Champlain *et al.*, 1980). Louis *et al.* (1973) further have observed a positive correlation between plasma norepinephrine concentration and diastolic blood pressure. An abnormality in tissue storage of norepinephrine resulting in an increase in circulating levels of the catecholamine and thus an increase in blood pressure has also been suggested (Mendlowitz *et al.*, 1965).

An increase in the activity of enzymes (such as dopamine β-hydroxylase) responsible for the synthesis of norepinephrine have been reported in hypertensive patients (de Quattro *et al.*, 1975). On the other hand, Louis *et al.* (1974) have demonstrated that elevated levels of norepinephrine are due to an enhanced sympathetic drive as opposed to an increase in the synthesis of norepinephrine.

Recent studies by de Champlain *et al.* (1981a, 1981b) have suggested that the sympathetic hyperreactivity observed in hypertensive subjects may actually be the result of a decreased inhibitory parasympathetic tone on sympathetic fibers.

The renin-angiotensin-aldosterone system has also been hypothesized to play a major role in the development of hypertension. Plasma renin concentrates are generally inversely related to blood pressure in normotensive individuals. Lucas *et al*. (1974) observed that 55 percent of untreated hypertensive patients had high plasma renin concentrations. This would imply that the normal negative feedback between renin and blood pressure was altered. Some investigators, though, have reported normal (Aurell *et al*., 1975) or even low (Brunner *et al*., 1972) plasma renin activity in hypertensive patients.

An increase in renin invariably leads to increased levels of angiotensin. Angiotensin can then have several effects which result in an increase in blood pressure. Besides having a direct vasoconstrictor effect, angiotensin can cause peripheral vasoconstriction via sympathetic nerves by stimulating central adrenergic components (Bickerton and Buckley, 1961), augmenting the synthesis and release of norepinephrine in sympathetic nerve endings (Malik and Nasjletti, 1976), and inhibiting norepinephrine uptake (Panisset and Bourdois, 1968). These responses appear to be greater in the hypertensive state.

Another hypothesis that has long been supported by Guyton involves a major role for the kidney. Guyton *et al*. (1972) have suggested that an increase in renal vascular resistance (but not total peripheral resistance) is a major cause of hypertension. This is supported by the observation that experimentally induced increase in renovascular resistance increases blood pressure but does not affect total peripheral resistance.

The preceding hypotheses take into account only one factor as an implicating component in essential hypertension. Page (1949) proposed the "mosaic theory" of hypertension to recognize the multifaceted nature of this disease process. It simply states that there are many mechanisms involved in blood pressure regulation and, therefore, hypertension could be the result of a combination of various abnormalities in the blood pressure regulating system.

Although no general agreement as to the cause(s) of hypertension has been reached, a common feature in essential hypertension, as well as experimental hypertension, is the increase in peripheral vascular resistance and alterations in the vascular smooth muscle itself.

Structural changes in the vaculature have been proposed as a primary contributor to the maintained increase in peripheral vascular resistance of the hypertensive state (Brody and Zimmerman, 1976). Bohr and Berecek (1976) contend that the increase in peripheral resistance is due to an increase in the sensitivity of the vasculature to vasoactive agents which causes the increase in blood pressure and eventually results in structural alterations. Folkow (1971), however, states that the increased wall thickness is an adaptive change which causes the increase in peripheral resistance. This adaptive response makes the vasculature hyperreactive, and, in addition, is responsible for the maintenance of hypertension after the initial hypertensive stimulus is removed.

Whether the alterations in vascular responsiveness are a result or the cause of the adaptive changes and increase in resistance, most investigators will agree that these alterations do occur. Studies on vascular smooth muscle from hypertensive patients and animals have demonstrated increased levels of sodium in the arterial wall (Tobian and Binion, 1954) and that these increased levels result in an enhanced responsiveness to various pressor agents (Harris, 1970).

Direct studies on the responsiveness of human vasculature, however, are scarce. Therefore, numerous experimental models of hypertension have been developed to investigate the alterations that occur in vascular smooth muscle in the hypertensive state. When compared to essential hypertension, though, each of these models have certain drawbacks.

The most appropriate model for essential hypertension is considered to be the spontaneously hypertensive rat (SHR). Through selective inbreeding of Wistar-Kyoto (WKY) rats with increased blood pressure, Okamoto and Aoki (1963) were able to develop the SHR. This provided conclusive evidence for a genetic factor in the genesis of hypertension; however, much controversy ensued over the contributing mechanisms for the development and maintenance of this form of experimental hypertension.

Another model considered to be a genetic form of hypertension is the Dahl rat. By selective inbreeding, Dahl et al. (1962a, 1962b) developed two substrains, one of which was sensitive to a high salt diet (Dahl-S) and developed hypertension, while the other was salt-resistant (Dahl-R). The results of these experiments clearly showed that hypertension induced by chronic salt treatment involved a genetic predisposition.

A number of renal hypertensive models have been developed also. Encapsulation of the kidneys with latex capsules results in a perinephritis and hypertension (Abrams and Sobin, 1947). Goldblatt et al. (1934) showed that an experimental form of hypertension which mimics clinical hypertension could be produced by restricting flow through the renal arteries. The technique involved placing a silver clip on the renal arteries of one kidney and removing the contralateral kidney.

Several other forms of experimental secondary hypertension include coarctation hypertension and hypertension induced by estrogen treatment.

Another form of experimental hypertension that has been studied quite extensively is the deoxycorticosterone-salt (DOC-salt) model (Selye et al., 1943). Administration of the mineralocorticoid DOC plus a 1 percent saline solution results in an increase in blood pressure. The development of hypertension in this model is dependent upon the high salt intake.

It was mentioned earlier that the role of sodium chloride (salt) in hypertension has become one of the major areas of study in the pathogenesis of this disease. It appears to be a major factor in the development of essential hypertension and is recognized as playing a role in most, if not all, forms of experimental hypertension.

The DOC-salt and Dahl-S models of hypertension, of course, require the presence of a high-salt diet. In addition, it has been shown that the development of hypertension in the SHR and estrogen-treated rat is accelerated with a high-salt diet. Hypertension in other models also appears to be exacerbated with a high-salt diet.

Recent evidence from studies on essential hypertensives have shown alterations in sodium and potassium co-transport mechanisms that could be used as an assay for determining essential hypertension (Dagher and Garay, 1980). In addition, Hamlyn _et al_. (1982) have shown the existence of a circulating inhibitor of sodium-potassium-ATPase (Na,K-ATPase) in essential hypertensive patients and suggest that it could play a role in the pathogenesis of this disease.

Evidence for alterations in sodium, potassium co-transport (Jones and Hart, 1975; Friedman and Friedman, 1976) and vascular Na,K-ATPase activity (Webb, 1982b), along with the presence of a circulating inhibitor of Na,K-ATPase (Pamnani _et al_., 1980a), has also been implicated in the pathogenesis of the DOC-salt model of hypertension.

It appears, then, that studies involving alterations in Na,K-ATPase activity and its role in the genesis of experimental hypertension would best be done in the DOC-salt model. Since there are similarities between this model of hypertension and essential hypertension, it may be possible to extrapolate the findings in studies using the DOC-salt model to essential hypertension.

PART II
GENERAL METHODS

Male Sprague-Dawley (S-D) rats were used in all experiments and were purchased from Blue Spruce Farms, Inc. (Altamont, NY). Animals were housed in groups of two in hanging stainless steel cages in a temperature regulated room (26± 1° C) which was illuminated from 0500 to 1900 hours. Unless otherwise specified, the animals were fed a commercial laboratory diet (Purina Laboratory Chow®, Ralston Purina Company, St. Louis, Missouri) and tap water ad libitum.

Mineralocorticoid hypertension was induced in S-D rats by administration of deoxycorticosterone acetate (DOCA; Sigma Chemical Company, St. Louis, Missouri) and 1 percent sodium chloride drinking solution. At 6 weeks of age, rats were anesthetized with ether, the right kidney removed and a pellet of DOCA (approximately 75 mg) implanted subcutaneously in the nape of the neck. These rats were fed a 1 percent sodium chloride drinking solution for the remainder of the experimental period (DOCA/NaCl). All studies on vascular reactivity were carried out at 4 weeks postimplant (10 weeks of age).

Systolic blood pressures were recorded from the tail of lightly anesthetized (ether) animals utilizing a pneumatic pulse transducer and a Physiograph Four-A (Narco

Bio-Systems, Houston, Texas). The average of three readings within 5 mm Hg of each other was recorded as the systolic blood pressure of that rat. Blood pressures, as well as body weights, were taken weekly and on the day of sacrifice (Figures 1 & 2).

To measure isometric contractions, all experiments on vascular reactivity were carried out using an F-50 microdisplacement myograph transducer and model DMP-4B physiograph recorder (Narco Bio-Systems).

At the time of experimentation, the animals were anesthetized with ether and each femoral artery quickly removed and placed in an aerated modified Krebs physiological solution at 26° C. Any fat, loose connective tissue, or blood was carefully removed. One 3 mm ring was cut from each femoral artery using a cutter consisting of two stainless steel blades mounted on an aluminum block 3 mm in width. The rings were then suspended between two stainless steel hooks (250 μm in diameter) inserted through the lunen (approximately 750 μm inside diameter) to record circular smooth muscle contraction. One hook was mounted to a bar that could be moved vertically to either increase or decrease force applied to the tissue. The other hook was attached to an F-50 microdisplacement myograph transducer to record isometric contractions.

Each isolated tissue was placed individually in a 20 ml double-jacketed muscle bath (Figure 3) containing modified Krebs solution. A circulation (Haake Buchler Instruments, Inc., Saddle Brook, NJ) and reservoir system maintained

Figure 1. Indirect systolic blood pressures of control and DOCA/NaCl hypertensive rats beginning one week prior to initiation of treatment until time of experimentation (4 weeks postimplant). Blood pressures were significantly greater ($p < .05$) in the DOCA/NaCl treated group at one week postimplant and rose to a level of approximately 190 mm Hg by 4 weeks postimplant ($p < .001$). Vertical bars represent one standard error of the mean.

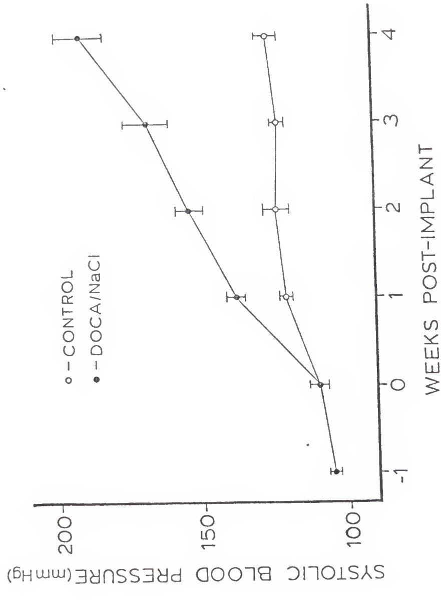

○ - CONTROL
● - DOCA/NaCl
SYSTOLIC BLOOD PRESSURE (mm Hg)
200
150
100
WEEKS POST-IMPLANT
-1
0
1
2
3
4

Figure 2. Mean body weights of control and DOCA/NaCl hypertensive rats beginning one week prior to initiation of treatment until time of experimentation (4 weeks postimplant). The average body weight of DOCA/NaCl treated rats was significantly less ($p < .01$) at the time of experimentation. Vertical bars represent one standard error of the mean.

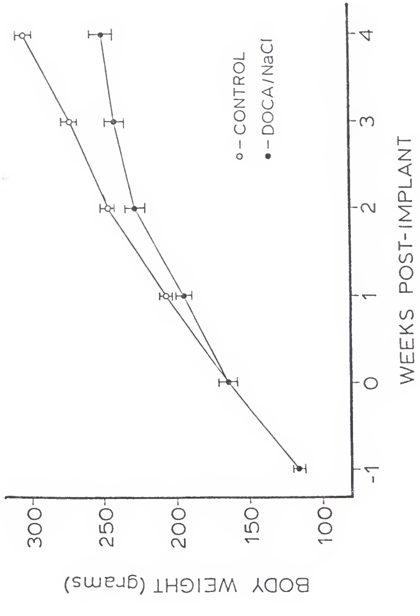
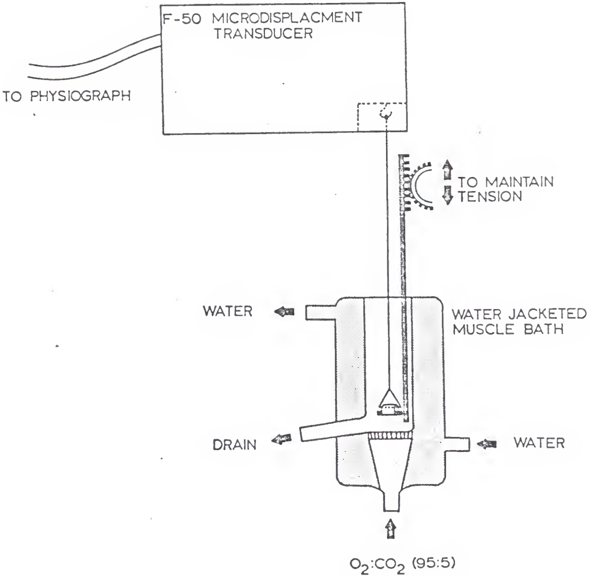

BODY WEIGHT (grams)
300
250
200
150
100
-1
0
1
2
3
4
WEEKS POST-IMPLANT
○ – CONTROL
● – DOCA/NaCl

Figure 3. Diagram of *in vitro* muscle bath.


F-50 MICRODISPLACMENT TRANSDUCER
TO PHYSIOGRAPH
TO MAINTAIN TENSION
WATER
WATER JACKETED MUSCLE BATH
DRAIN
WATER
O_2:CO_2 (95:5)

the temperature of the Krebs solution at 37° C. The Krebs solution was aerated with an O_2/CO_2 (95:5) gas mixture which maintained the pH at 7.3.

After removal of the femoral arteries, the animals were sacrificed by decapitation and blood collected from the trunk region into 7 ml disposable test tubes. The blood was allowed to clot for approximately 1 hour after which the clot was removed from the test tube. The remaining serum was centrifuged (Trias, Clay Adams) for 5 minutes at 3500 rpm. An aliquot of the serum was placed into 1.5 ml Eppendorf Micro Test tubes (Brinkman Industries) and kept frozen at -20° C until time of analysis. After collection of blood, the heart, kidneys, adrenals, thyroid, and spleen were removed, cleaned of fat, blotted dry and weighed. Weights were expressed as mg/100gm of body weight (Table 1).

Unless otherwise specified the composition (mM) of the modified Krebs solution in double-distilled water was NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgCl_2$, 1.2; $NaHCO_3$, 12.5; dextrose, 11.5; disodium EDTA, 0.01.

Solutions of Krebs and solutions of norepinephrine ((-)-Arterenol HCl, Sigma), dl-isoproterenol HCl (Sigma), sodium nitrite (Sigma), serotonin (5-hydroxytryptamine creatinine sulfate, Sigma), ouabain octahydrate (Sigma), and phentolamine HCl (Regitine®, Ciba-Geigy) were prepared fresh each day. Volumes of these drugs added to the 20 ml muscle bath ranged from 0.01 to 0.20 ml.

Experiments on vascular reactivity were carried out as follows, unless otherwise specified. After mounting the tissues in a muscle bath, the rings of femoral artery were equilibrated for 90 minutes at a predetermined preload force (baseline). During the equilibration period the Krebs solution was changed every 15 minutes. Following the 90 minute equilibration period an accumulative potassium chloride (KCl) dose-response curve (8 to 80 mM) was obtained (Van Rossum, 1963). Each concentration was allowed to remain in contact with the tissue for a 3 minute period of time. After the last addition of KCl, 20 ml of fresh Krebs solution was added to each bath and a 15 minute washout curve generated. The time (seconds) to decrease to 50 percent of the maximal response was recorded ($t_{\frac{1}{2}}$ value). During the washout curves, tissues were allowed to return to baseline. If any tissues relaxed below the baseline value before the end of the 15 minute washout period, the preload force was adjusted to bring the tissues back to baseline. At the end of the 15 minute washout period, the Krebs solution was changed again and the tissues kept at baseline for an additional 15 minutes.

Following this additional 15 minute washout period, fresh Krebs solution was added to each bath along with sufficient KCl to produce approximately 50 percent contraction as determined from the previous KCl dose-response curve. The contractions were allowed to plateau, at which time an accumulative isoproterenol (ISO) dose-response curve (10^{-9} to 10^{-4} M) was obtained (Cohen and Berkowitz, 1974). Each concentration of isoproterenol was allowed to remain in contact with the tissues for a 3 minute period of time. Following the last dose of ISO, an accumulative dose-response curve for sodium nitrite ($NaN0_2$; 1.5×10^{-5} to 1.5×10^{-3} M) was instituted. This was done without changing the bath solutions. Each concentration of $NaN0_2$ was allowed to remain in contact with the tissue for a 3 minute period of time. After the last dose of $NaN0_2$, the drugs were washed out, fresh Krebs solution added and the tissues allowed to return to baseline. The tissues were maintained at baseline and the Krebs solution changed every 15 minutes for a period of 1 hour.

Following the 1 hour wash period, an accumulative norepinephrine (NE) dose-response curve (10^{-10} to 10^{-4} M) was done. Each concentration of NE was allowed to remain in contact with the tissue for a 5 minute period of time.

At the conclusion of each experiment the tissues were removed, allowed to dry and weighed to a constant weight on a Cahn electrobalance. Physical and hemodynamic values for control and DOCA/NaCl hypertensive rats at the time of experimentation are given in Table 1.

Data for the KCl and NE dose-response curves were expressed as grams of force per milligram of dry tissue weight (active tension). However, expressing the data in this manner can result in differences in the maximum contractile response observed when comparing treatments.

TABLE 1. PHYSICAL AND HEMODYNAMIC VALUES OF CONTROL AND DOCA/NaCl TREATED RATS AT 4 WEEKS POSTIMPLANT.

Treatment Group	Body Weight (g)	Systolic Blood Pressure (mm Hg)	Wet Organ Weight/Body Weight Ratios (mg/100 g body weight)					Femoral Ring Weight (mg; dry weight)	DOCA (mg/kg/day)
			Heart	Kidneys	Adrenals	Thyroid	Spleen		
Control	305 ± 5[a]	126 ± 4	277.81 ± 1.70	709.04 ± 5.03	14.80 ±0.45	4.80 ±0.16	217.08 ±10.87	0.1863 ±0.0065	--
DOCA/ NaCl	250 ± 5[c]	191 ± 8[d]	388.19[d] ± 6.42	988.48[c] ±23.64	17.65[b] ±0.40	5.95[b] ±0.29	263.08[b] ±14.81	0.2097[c] ±0.0070	2.41 ± 0.14

[a] Mean ± SEM

[b] Significantly different from control ($p < .05$)

[c] Significantly different from control ($p < .01$)

[d] Significantly different from control ($p < .001$)

To account for these differences in the responsiveness of vascular smooth muscle to either KCl or NE, the data for each drug (at each concentration) were normalized and expressed as a percent of the maximum active tension generated for that agent.

The relationship between the dose of ISO or $NaNO_2$ administered and the response ellicited is expressed in a different manner. As already stated, the tissues are precontracted with KCl and the tension at which they stabilize is considered the 100 percent value. Any changes in tension during either the ISO or $NaNO_2$ dose-response curves are expressed as a percent of KCl contraction.

Certain terms are used to describe the responsiveness of vascular smooth muscle to vasoactive agents. In this dissertation, sensitivity will be used to describe the response of vascular smooth muscle to low doses of a drug. Contractility will refer to the maximum active tension generating capability of the smooth muscle to high doses of a drug. Another measure of sensitivity is the effective dose (ED) which produces a certain degree of response. In this dissertation, sensitivity of vascular smooth muscle to a drug will also be expressed as ED10 (the effective dose required to produce a response equal to 10 percent of the maximal response).

Pellets of DOCA were made using an F.J. Stoker tablet maker. Determination of the amount of DOCA received by the animal during the experimental period was calculated as follows: the pellets were placed in a desiccator for 72 hours and then weighed on an analytical balance prior to subcutaneous implantation. At the time of sacrifice, the pellets were removed, cleaned of any adhering tissue and placed in a desiccator for 72 hours. The pellets were then reweighed on the same balance. The value derived from the difference in pre and postexperimental weights was divided by the mean body weight of the animal during the time the pellet was implanted. The dosage was expressed at mg/kg/day of DOCA (Table 1).

Students t-test was used to determine statistical significance between two groups. When more than two groups were analyzed an analysis of variance (ANOVA) was used. The identification of significant differences between the means of two groups was made by the use of the Student-Newman-Kuels test. Significance was set at the 95 percent confidence limit.

PART III
ALTERED VASCULAR REACTIVITY IN DOCA/NaCl HYPERTENSIVE RATS: ROLE OF CALCIUM

Introduction

Despite years of investigation and numerous studies, a general agreement as to the cause(s) of essential hypertension has not been reached. One of several initiating factors may be involved in the pathogenesis of hypertension. These initiating factors may then activate any one or more of a sequence of events involving neurogenic, endocrine, renal, and/or cardiovascular changes. Whatever the cause, essential hypertension, as well as all forms of experimental hypertension, follows a final common pathway, that being vascular smooth muscle (VSM) changes resulting in the increase in peripheral vascular resistance.

Two major components of the change in VSM during hypertension include morphological changes as well as alterations in vascular reactivity. Most investigators will agree that both changes occur; however, much controversy still exists over whether the alterations in vascular reactivity precede or follow the structural changes.

As early as 1938, Karsner reported finding at autopsy a thickening of the aortic media of persons who had hypertension. Vascular structural changes have also been reported in several forms of experimental hypertension, such as the spontaneously hypertensive rat (SHR) (Folkow et al., 1974; Warshaw et al., 1979), rats made hypertensive with deoxycorticosterone acetate (DOCA) (Wolinsky, 1970), and renal hypertensive rats (Lundgren, 1974). Folkow et al. (1974) further stated that the hypertrophic vascular changes seen in the SHR are confined to the precapillary resistance vessels. More recently, however, Lee et al. (1983a) showed that in the SHR hypertrophy of the medial layer occurred not only in the small arteriolar vessels, but also in large elastic and muscular arteries. More interestingly, Greenberg et al. (1978) and Greenberg and Wilborn (1982) observed a smooth muscle hypertrophy of the portal vein, inferior vena cava, and pulmonary artery of SHR, and suggested that a circulating humoral factor increases in hypertension and acts on both veins and arteries to initiate structural changes. In opposition to these findings, Mulvany et al. (1980b) found no difference in morphology between portal veins from SHR and Wistar Kyoto controls.

In addition to hypertrophy, an increase in VSM wall thickness has been attributed to hyperplasia of smooth muscle cells (Freis et al., 1972). Hyperplasia has been reported by Bevan et al. (1976) in experimental hypertension in the rabbit and by Warshaw et al. (1979) and Fischer (1976) in the SHR.

Significant increases in elastin and collagen have been reported also in the aorta of DOCA-hypertensive rats (Wolinsky, 1970). Furthermore, Wolinsky (1971) was able to show that amounts of elastin and collagen remained elevated after the reversal of renal hypertension.

Folkow *et al*. (1970,1973) have long been major proponents of the hypothesis that the alterations in vascular reactivity and increases in peripheral resistance are due to the adaptive structural changes observed in the hypertensive state. Mulvany *et al*. (1980a) also have shown that the increased sensitivity of resistance vessels of the SHR is due to a decreased lumen to wall thickness ratio. In support of Folkow's adaptation theory, Henrichs *et al*. (1980) observed that arterial media hypertrophy is a result of high blood pressure and not a genetically pathologic factor. More recently, however, Gray (1982) has presented evidence suggesting that arterial wall hypertrophy may be genetically determined to some extent in the SHR since mean arterial pressure and the ratio of wall thickness/lumen radius were significantly higher in one-day-old pups.

Some investigators theorize that the structural alterations observed in the hypertensive state are secondary to the causes of hypertension. This theory is based upon the premise that the increase in peripheral resistance is due to a hypersensitivity of the VSM to circulating vasoconstrictors.

Bolli et al. (1981) and Amann et al. (1981), conducting studies in essential hypertensive patients, showed an enhanced α-adrenergic mediated vasoconstriction in response to various tests. Numerous studies have been done in experimental models of hypertension indicating that VSM responsiveness to various vasoactive agents is increased in hypertension. Hypersensitivity of VSM has been observed in femoral artery strips of DOCA-hypertensive rats (Bohr and Sitrin, 1970; Holloway and Bohr, 1973; Webb, 1982a), renal hypertensive rats (Bandick and Sparks, 1970) and SHR (Holloway and Bohr, 1973). Field et al. (1972) observed an increase in the response of rings of aortic smooth muscle from SHR and renal hypertensive rats to low doses of norepinephrine. Mulvany and Nyborg (1980) obtained similar results with segments of mesenteric resistance vessel. Using an intact perfusion technique, Lee et al. (1980) observed a significantly greater increase in vascular resistance of SHR in response to infusions of norepinephrine and tyramine supporting the contention that the elevated vascular resistance in SHR may be attributed to a vasoconstrictor hyperresponsiveness to catecholamines. Similar results have been obtained using isolated perfused vessels from SHR (Mulvany and Halpen, 1977), renal hypertensive rats (Collis and Alps, 1977), and DOCA-hypertensive rats (Collis, 1981).

Several investigators, however, contend that there is no hypersensitivity of the VSM. Noon et al. (1977), Fitzpatrick and Szentivanyi (1980), Aoki et al (1980) and Hallbäck et al. (1971) using strips of thoracic aorta from SHR observed a decrease in the maximal contractility in response to norepinephrine. Aortic smooth muscle from rats with aortic coarctation hypertension also exhibited a decrease in the maximal response to norepinephrine, serotonin, and potassium chloride (Lai et al., 1980). Contractile responses of aortic strips from DOCA-hypertensive rats to norepinephrine showed a time-dependent decrease which paralleled the increase in blood pressure (Pang and Sutter, 1980). Hermsmeyer et al. (1982) saw no difference in the norepinephrine response of strips of caudal artery from DOCA-hypertensive or Dahl genetic hypertensive rats when compared to controls. In support of this are the recent findings of Smith and Jones (1983) in which Dahl hypertensive rats showed aortic hypertrophy with no increase in the sensitivity to norepinephrine stimulation.

Using digital arteries obtained postmortem from hypertensive patients, Moulds (1980) showed a decrease in the maximal response to norepinephrine and serotonin.

In order to more clearly elucidate the time course of alterations in vascular reactivity and morphology and determine which appears to play the major role in the increase in peripheral vascular resistance, several longitudinal studies using various models have been done.

Using an animal model in which one femoral artery was protected from high blood pressure while the other was exposed to the increased pressure, Hansen and Bohr (1975) observed no difference in the sensitivity and contractility of femoral artery strips to epinephrine. SHR and DOCA-hypertensive rats were used in their study to show that the lack of the increase in wall stress did not prevent an increase in the sensitivity of VSM to adrenergic stimuli. Berecek et al. (1980) observed a significant increase in vascular reactivity of isolated perfused kidneys from DOCA-hypertensive rats to norepinephrine, angiotensin II, and vasopressin, prior to an increase in blood pressure. A more recent study supports this role for an increase in vascular reactivity as a major causative factor for the increase in blood pressure. Katovich et al. (in press) observed an increase in the sensitivity of aortic rings to norepinephrine prior to an increase in blood pressure in intact DOCA-hypertensive rats. In a different form of experimental hypertension, two-kidney, one-clip Goldblatt hypertensive dogs, Greenberg (1981) observed an increase in vascular reactivity prior to an increase in arterial resistance.

In opposition to these findings, Cheng and Shibata (1980) suggest that the increase in blood pressure, per se, contributes to the enhanced vascular reactivity seen in the SHR since occlusion of the abdominal aorta resulted in a decrease in the responsiveness of isolated perfused hindquarters to norepinephrine, serotonin, and potassium chloride.

Mueller (1983) conducting a longitudinal study on SHR and Dahl hypertensive rats saw no difference in the norepinephrine sensitivity of either group when compared to controls at 5, 11, and 17 weeks of age. Assessment of structural alterations, however, revealed that an increase in smooth muscle mass was already present at 5 weeks of age. Although Mulvany et al. (1980a) saw no difference in media thickness of mesenteric resistance artery from SHR at 6 weeks of age, an increase did occur between 6 and 24 weeks of age. In addition, they also observed an increase in norepinephrine sensitivity of the resistance vessels.

Finch (1974) found that treating SHR with hydralazine, hydrochlorothiazide and reserpine from 16 to 20 weeks of age reduced their blood pressure but did not alter reactivity to norepinephrine. Hamilton (1975), on the other hand, treated SHR prior to the onset of hypertension and saw that the increase in reactivity of perfused preparations was prevented. From this, Mulvany (1983) concluded that both structural and reactivity abnormalities may be present before the onset of hypertension and, therefore, both may be important in the development and maintenance of hypertension.

Most investigations dealing with alterations in vascular reactivity of hypertensive animals involve changes in the responsiveness to vasoconstrictor stimuli, norepinephrine in particular. Few studies have been done on the β-adrenergic component of the vasculature of hypertensive rats. The results of these studies, however, are conflicting.

Godfraind and Dieu (1978) found that relaxation of rings of aortic smooth muscle from SHR was decreased compared to controls. Cohen and Berkowitz (1976) and Cheng and Shibata (1981) observed similar results in aortic strips from SHR and renal hypertensive rats. Asano et al. (1982) showed that β-receptor mediated norepinephrine-induced relaxation as well as isoproterenol-induced relaxation was attenuated in femoral artery strips from SHR. In contrast, Deragon et al. (1978) reported an increased sensitivity to β-adrenergic stimulation in perfused hindlimbs of SHR. Winquist and Bohr (1983) observed no differences in the response of basilar arteries from SHR to isoproterenol when compared to controls.

Other measures of β-adrenergic mediated responses also have shown alterations in this system in hypertensive animals. Isoproterenol-induced cyclic-AMP accumulation in thoracic aortic rings from SHR has been shown to decrease between 7 and 18 weeks of age (Schoeffter and Stoclet, 1983).

Increases in water intake and plasma renin activity following isoproterenol administration were considerably less in renal hypertensive rats when compared to controls, suggesting a decrease in β-adrenergic-mediated responses in the renal hypertensive rat (Katovich and Fregly, 1983).

Woodcock et al. (1980) observed a decrease in the number of β-adrenoceptors in mesenteric arteries of DOCA-hypertensive rats using radioligand binding studies. In addition, recent work by Katovich et al. (1984) has shown that isoproterenol-induced relaxation of aortic rings from DOCA/NaCl treated rats decreases with a concomitant increase in blood pressure, suggesting that the vascular β-adrenergic system may play an important role in the increase in peripheral vascular resistance of the DOCA-hypertensive rat.

It is apparent that alterations in both α- and β-adrenergic responsiveness occur in VSM from hypertensive animals which may contribute to the increase in peripheral vascular resistance. However, the mechanism(s) behind these alterations remain to be elucidated. One factor that is common to both systems, and is known to play a key role in many cellular processes, is the calcium ion (Ca^{++}). Many studies have been done on the role of Ca^{++} in excitation-contraction (membrane depolarization) and pharmacomechanical coupling (contraction with little or no change in membrane potential) in VSM from normotensive animals as well as alterations in these responses in VSM from hypertensive animals. Although numerous studies have been done investigating β-adrenergic relaxation and Ca^{++} sequestration in VSM from normotensive animals, no studies have been done on the role of Ca^{++} in the alterations seen in β-adrenergic responsiveness of VSM from hypertensive animals. To maintain myogenic tone the intracellular levels of Ca^{++} must be maintained at a concentration of approximately 10^{-7} M. Upon stimulation by a vasoactive substance the intracellular levels of Ca^{++} rise to approximately 10^{-5} to 10^{-4} M.

The source(s) of Ca^{++} available to activate the contractile apparatus of VSM may be intracellular, extracellular, or both depending upon the type of vascular muscle and the nature of the stimulus.

It has been suggested that the alteration in the reactivity of vessels from hypertensive animals may be the result of a change in the way in which Ca^{++} is handled by the VSM cell. One of these is an increase in the permeability of the VSM cell membrane to Ca^{++} (Hinke, 1966; Holloway et al., 1972; Holloway and Bohr, 1973; Noon et al., 1978). Fitzpatrick and Szentivanyi (1980) hypothesize that if there is an increase in permeability to Ca^{++} then the VSM could well exist in a state of elevated myogenic tone. They tested this hypothesis by incubating aortic strips from both SHR and Wistar-Kyoto (WKY) rats in physiological solutions with varying concentrations of Ca^{++}. The strips from the SHR exhibited a Ca^{++}-dependent decrease in tone upon exposure to decreasing concentrations of Ca^{++} ranging from 2.5 to 0 mM while strips from the normotensive rats showed no response. Readdition of Ca^{++} in increasing concentrations resulted in a concentration related increase in tension of the strips from SHR. The response of SHR aortic strips to high concentrations of norepinephrine plus Ca^{++} after incubation in a Ca^{++} free medium was similar to WKY aortic strips, whereas this response was less in the SHR when carried out in normal Ca^{++} physiological solution. This observation can be explained in terms of an alteration in Ca^{++} permeability. In a Ca^{++}-free media the tissues are initially equivalent with respect to tension since this procedure decreased intracellular Ca^{++} to very low levels. Adding an agonist simultaneously with Ca^{++} results in an immediate response which is similar for both the WKY and SHR vessels. If the tissues are allowed to equilibrate in normal Ca^{++} physiological solution the increased permeability of the SHR vessel to extracellular Ca^{++} actually results in a higher level of myogenic tone when compared to the WKY vessel. However, this difference is not corrected for and, therefore, when the agonist is added the response that is observed is less than that of the WKY vessel. SHR aortic strip responsiveness was increased at low concentrations of norepinephrine in normal physiological solution when compared to WKY. However, when the strips were normalized at 0 mM Ca^{++} the responses were similar. Thus, it would appear that the increase in sensitivity and the decrease in contractility seen in vessels from hypertensive animals may be due to an increased myogenic tone as a result of the increase in permeability to Ca^{++}. Aoki _et_ _al_. (1981) incubated strips of aortic smooth muscle from SHR and WKY in an EGTA/Ca^{++}-free medium and observed that after the incubation period a Ca^{++}-induced contraction of the strips from SHR was greater than that of the WKY. These results also suggest an increase in Ca^{++} permeability of the VSM cell membrane.

Using mesenteric resistance vessels from SHR, Mulvany and Nyborg (1980) reported no difference in potassium (potential)-dependent Ca^{++} permeability when compared to controls; however, norepinephrine-induced Ca^{++} permeability was abnormally high. In contrast, Pedersen et al. (1978) observed markedly reduced contractions of SHR aortae in a Ca^{++}-free medium in response to both potassium and norepinephrine. They suggested that, in the SHR, contraction induced by norepinephrine and potassium is more dependent on extracellular Ca^{++} than is the response of controls. Pang and Sutter (1981) obtained similar results using low Ca^{++} (0.2 and 0.4 mM) solutions. Based upon studies using isolated perfused hindquarters, Folkow et al. (1977), however, conclude that the increase in vascular hyperreactivity of VSM from SHR is not the result of an alteration in the way in which Ca^{++} is handled by the VSM cell.

Mulvany et al. (1981), on the other hand, suggest that the increased Ca^{++} sensitivity of resistance vessels in SHR is an intrinsic defect independent of blood pressure and neurogenic influences since antihypertensive treatment reduced blood pressure and eliminated the increase in vessel media thickness, but did not reduce norepinephrine- and Ca^{++} sensitivity.

Recent evidence (Wei et al., 1976; Kwan et al., 1980; Kwan and Daniel, 1981) has shown that the calcium gradient (defined as ATP-dependent accumulation prevented by ionophores) across VSM membranes is decreased in SHR, one-kidney one clip, and two-kidney one clip hypertensive rats. This reduced gradient can be attributed to either an increase in calcium permeability or a decrease in calcium pump activity of the cell membrane. This may provide a reasonable explanation for the observed increase in the calcium content of mesenteric arterioles (Tobian and Binion, 1954) and a slower rate of relaxation of aorta (Field et al., 1972) from hypertensive rats.

In addition, it appears that these alterations in membrane Ca^{++} handling by VSM from SHR are not solely a consequence of the increase in blood pressure since anti-hypertensive treatment did not reverse the alterations (Kwan and Daniel, 1982).

Wright and Rankin (1982) and Wright et al. (1980) propose that, even though levels of ionic calcium in whole blood are decreased in male SHR and DOCA/salt hypertensive animals, this disturbance in calcium metabolism is probably not a causal factor in the development of hypertension, since female SHR and other forms of renal hypertension show no alteration in ionic or total blood calcium. However, these slight alterations may have some effect on increasing VSM sensitivity to constricting agents since McCarron et al. (1981) observed that SHR fed a high calcium diet (4 percent) beginning at 10 weeks of age resulted in a normalization of serum ionized calcium and attenuated the development of hypertension in the SHR.

From the preceding discussion it is evident that there is some controversy regarding the responsiveness of VSM from hypertensive animals when compared to normotensive animals. Some investigators have indicated that a hyperresponsiveness of VSM from hypertensive animals to circulating catecholamines exists, while others contend there are no differences. In addition, much controversy remains over whether the increase in vascular reactivity is a major factor in the initiation of hypertension as opposed to structural alterations.

Variations in the results may be due to a number of factors, such as the age, sex, and strain of animal used as well as the use of proper control animals. Differences in the experimental design also lead to variations in the interpretation of results.

In terms of isolated VSM and its use in studies on vascular reactivity, one major problem in experimental design that may contribute to the variations in responsiveness is the length or force at which a tissue is incubated. It is known that a length-tension relationship exists for skeletal muscle. The strength of isometric contraction that occurs depends upon the amount of initial preload force or stretch on the muscle. Strips as well as rings of VSM also have been shown to have a length-tension relationship (Roach and Burton, 1959; Horowitz, *et al.*, 1974; Price *et al.*, 1981). This concept of length-tension may possibly be used to explain some of the variability in reports on vascular reactivity. One possible error that may exist is that in *in vitro* experiments, all tissues are subjected to the same amount of preload force, whereas *in vivo*, the forces may be different. Gordon and Nogueira (1961) performed length-tension experiments on aortic strips from normotensive and renal hypertensive rats. The aortic strips from the hypertensive animals required a higher initial preload tension to develop maximal active tension. According to the law of LaPlace (tension = pressure x radius) a vessel taken from a hypertensive animal should be subjected to a higher preload tension (or force) *in vitro* than a normotensive vessel since the vasculature of a hypertensive animal is subjected to higher mean pressures.

Another possible source of error involves the way in which results are reported. Some investigators quantify a response (contraction) as milligrams or grams of force per strip or ring. Since all strips or rings may not weigh the same, it is likely the responses will not be the same. One strip or ring may develop more force than another due to the presence of more contractile tissue, such as in a hypertensive animal. Therefore, the tissue should be weighed or cross-sectional area determined at the end of each experiment and contractions should be expressed as grams of force per weight or cross-sectional areas of tissue. This would normalize the responses between tissues and allow for appropriate statistical analysis.

A third possible source of error that may result in differences of responsiveness is the type of tissue preparation used. Numerous investigators use strips of VSM; however, the way in which the strip is cut can greatly affect the maximal response. Herlihy (1980) demonstrated that variations in the width of the strip, the vessel diameter, and pitch of the helix can lead to significant alterations in the tension-generating capacity of VSM. In addition, he also observed that strips cut perpendicular to the longitudinal axis developed the greatest amount of tension. Hansen et al. (1980) obtained similar results using strips of aortae from DOCA-hypertensive rats cut at various angles. Using computer assisted analysis, Todd et al. (1983) observed that 21 percent of the VSM cells in femoral artery are arranged circumferentially. The remaining cells take on a clockwise (17 percent) or counterclockwise (62 percent) configuration at only a 9 degree angle from the transverse axis. Therefore, in terms of the femoral artery and based upon previous findings, it appears that the angle at which strips of femoral artery should be cut, which results in a maximal response with minimum damage to the VSM cells, is at 90 degrees to the longitudinal axis. In other words, a ring of VSM would be most appropriate for studying alterations in vascular reactivity of hypertensive animals.

In the first part of this dissertation, experiments will be reported which determined the preload force at which rings of femoral artery from control and DOCA-hypertensive rats maximally respond. This preload force will be used in subsequent experiments designed to investigate alterations in vascular reactivity of DOCA-hypertensive rats to potassium chloride (a nonspecific smooth muscle stimulant), isoproterenol (a β-adrenergic agonist), sodium nitrite (a nonspecific smooth muscle relaxant), and norepinephrine (an α-adrenergic agonist). Finally, the first part of this dissertation will examine the role of extracellular calcium ion in VSM responsiveness to the above vasoactive agents and its importance in the alterations that occur in vascular reactivity of DOCA-hypertensive rats.

Experiment I

This experiment was designed to determine the preload force (in grams) at which maximum active tension develops in rings of femoral artery from control and DOCA/NaCl hypertensive rats

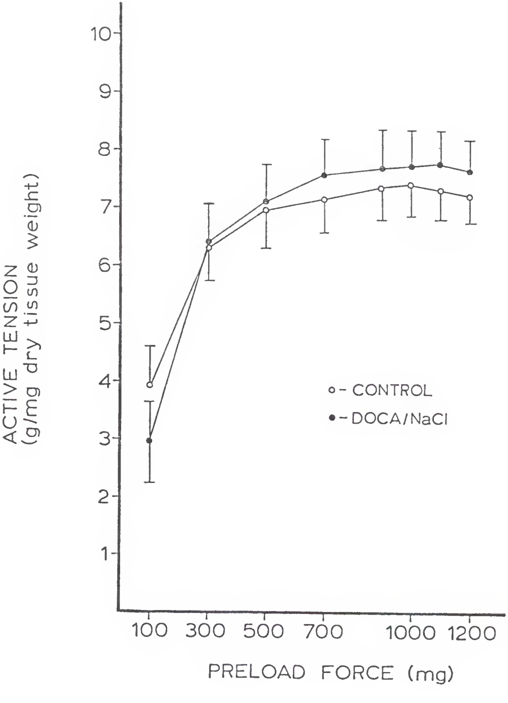
Methods

Twelve 6 week-old male S-D rats were used in this experiment. Deoxycorticosterone acetate/sodium chloride hypertension was induced in 6 of these rats as described in the General Methods section. At 10 weeks of age (4 weeks postimplant) all animals were sacrificed and rings of femoral artery prepared as described in General Methods. A force-tension curve was then generated as follows. Rings of femoral artery were equilibrated for 90 minutes at an initial preload force of 0.1 gram. Following the equilibration period, tissues were maximally contracted with 60 mM KCl. The tissues remained in contact with the KCl for a 15 minute period of time after which the KCl was washed out and the tissues allowed to return to baseline. The active tension generated at the end of the 15 minute period was recorded. Fresh Krebs solution was added to each bath and the initial (baseline) preload force increased by 0.2 gram. The tissues were allowed to equilibrate for 15 minutes at this new preload force before another addition of KCl. This procedure was repeated through 0.9 gram, after which the preload force was increased by an increment of 0.1 gram. This was done through 1.2 grams of preload force.

Results

Development of active tension by rings of femoral artery from control and DOCA/NaCl hypertensive rats increased as preload force was increased (Figure 4). Maximum active tension was slightly greater in the DOCA/NaCl treated group (7.75 g/mg dry tissue weight) and occurred at 1.1 gram of preload force. Active tension decreased as preload force

Figure 4. Effect of increasing preload force on development of active tension by rings of femoral artery from control and DOCA/NaCl hypertensive rats in response to 60 mM potassium chloride stimulation. Each point represents the mean and standard error of 12 rings.


ACTIVE TENSION
(g/mg dry tissue weight)
10
9
8
7
6
5
4
3
2
1
100
300
500
700
1000
1200
PRELOAD FORCE (mg)
○ - CONTROL
● - DOCA/NaCl

was increased beyond 1.1 gram. In femoral smooth muscle from the control group, maximum active tension (7.34 g/mg dry tissue weight) was generated at a preload force of 1.0 gram. As with the DOCA/NaCl group, active tension decreased as preload force was increased beyond 1.0 gram in controls. Femoral ring weights of control and DOCA/NaCl hypertensive rats were 0.2054 ± 0.0046 mg and 0.2166 ± 0.0048 mg, respectively.

Discussion

This experiment was designed to determine the preload force at which maximum active tension is generated by rings of femoral artery from control and DOCA/NaCl hypertensive rats. Since alterations in responsiveness may be due to the preload force or length at which VSM is incubated, it was important to determine this value in both groups for subsequent studies on vascular reactivity.

Although rings of femoral artery from the DOCA/NaCl group exhibited a maximal response at a higher preload force than controls (1.1 vs. 1.0 gram), all subsequent experiments were carried out at 1.0 gram of preload force in both groups. This value was chosen for both groups due to the fact that in the range from 0.9 gram to 1.1 gram of preload force little difference was observed in the maximal response to KCl stimulation in either group.

Rings of femoral artery from DOCA/NaCl hypertensive rats showed a greater response (although not significant) than controls at preload forces of 0.3 gram to 1.2 gram; however, the response at 0.1 gram was less. This could possibly be explained by the differences observed in femoral ring weights. The rings of femoral artery from DOCA/NaCl hypertensive rats weighed slightly more than controls, suggesting possible structural changes in the VSM. Possible structural alterations can include either hypertrophy or hyperplasia of the smooth muscle cells as well as changes in the content of elastin and collagen.

At the low preload forces, rings of femoral artery from controls would actually be stretched to a greater degree than VSM from DOCA/NaCl hypertensive rats due to the lesser amount of smooth muscle cells and/or collagen and elastin. Therefore, upon stimulation, the VSM from controls would respond more. At higher preload forces, though, the VSM cells from DOCA/NaCl hypertensive rats are stretched to a level at which they can maximally respond. In this case VSM from DOCA/NaCl hypertensive rats respond more than controls at the higher preload forces. This may be due to a greater number of smooth muscle cells (hyperplasia) that can be activated. Lee et al. (1983b) have observed hypertrophy of endothelial cells, as well as hypertrophy and hyperplasia of smooth muscle cells in VSM from 10-12 week old SHR. In addition, there was an increase in elastin, but no difference in collagen.

As in the SHR, increases in endothelial and subendothelial cells have been observed in DOCA/NaCl hypertensive rats after 4 weeks of treatment (Haudenschild et al., 1981). It has been suggested that the increase in arterial mass of DOCA/NaCl hypertensive rats is mainly the result of cellular hyperplasia (Mangiarua et al., 1981). This is supported by the findings of Cox (1982) who showed no alterations in the content of elastin and collagen of aorta as well as carotid and tail arteries from DOCA/NaCl treated rats. These observations also were made after 4 weeks of treatment.

Based upon these results, the increase in femoral artery ring weight from DOCA/NaCl hypertensive rats observed in this study is most likely due to a hyperplasia of VSM cells. The increase in the number of smooth muscle cells results in an increase in the maximal response. However, the hyperplasia does not appear to appreciably affect the preload force at which maximum active tension occurs. Hansen et al. (1974) obtained similar results using strips of femoral artery from DOCA/NaCl hypertensive rats. Cross-sectional areas of VSM from the DOCA/NaCl treated rats were slightly greater than controls. Length-tension studies also showed that strips of femoral artery from control and DOCA/NaCl hypertensive rats responded maximally at the same length.

Experiment II

This experiment was designed to investigate alterations in vascular smooth muscle responsiveness of the DOCA/NaCl hypertensive rat to various agonists. Tissues were equilibrated and dose-response curves carried out at the preload force determined in Experiment I.

Methods

Part A

Twelve 6 week-old male S-D rats were used in this experiment. Deoxycorticosterone acetate/sodium chloride hypertension was induced in 6 of these rats as described in the General Methods section. At 10 weeks of age (4 weeks postimplant) all animals were sacrificed, rings of femoral artery prepared, and accumulative dose-response curves to KCl, ISO, $NaNO_2$, and NE carried out as described in General Methods.

Part B

An additional 12 male S-D rats were used for this experiment. Again, 6 were made hypertensive with administration of DOCA and 1 percent NaCl. At the appropriate time the animals were sacrificed, femoral arteries prepared and dose-response curves to KCl, ISO and $NaNO_2$ carried out on rings of femoral smooth muscle as described in General Methods. However, one ring from each animal was incubated in phentolamine (10^{-5} M) for 15 minutes prior to the KCl and ISO dose-response curves.

Results

Part A

A significant increase in the response of femoral smooth muscle from DOCA/NaCl hypertensive rats to KCl at concentrations of 8 to 40 mM was observed (Figure 5). Although rings of femoral smooth muscle from the DOCA/NaCl treated group exhibited an overall greater maximum active tension (10.12 vs. 9.16 g/mg dry tissue), no significant difference was observed at 60 and 80 mM. Expressing the data as a percent of the maximal KCl response (Figure 6) shows that a significant difference still remains at KCl concentrations of 8 to 40 mM. The $t_{\frac{1}{2}}$ values for KCl washout were 327 ± 73 second and 30 ± 2 seconds for DOCA/NaCl and controls, respectively ($p < .001$).

Relaxation of femoral smooth muscle, expressed as a percent of KCl contraction, in response to ISO and $NaN0_2$ is shown in Figure 7. ISO-induced relaxation of rings of femoral smooth muscle from DOCA/NaCl treated rats was significantly attenuated when compared to controls. The initial response to ISO occurred at 10^{-7} M for both groups (2 percent relaxation for DOCA/NaCl vs. 14 percent relaxation in controls). Maximal relaxation in the DOCA/NaCl group was observed at 10^{-6} M (9 percent relaxation) while rings of femoral artery from control animals responded maximally at 10^{-5} M (48 percent relaxation). An increase in tension of femoral smooth muscle from DOCA/NaCl hypertensive rats occurred at 10^{-5} and 10^{-4} M ISO. This increase

Figure 5. Dose-response relationship between concentration of KCl and development of active tension by rings of femoral artery from control and DOCA/NaCl hypertensive rats. The response of vascular smooth muscle from DOCA/NaCl hypertensive rats to KCl (8 to 40 mM) was significantly greater ($p < .001$) than controls. Each point represents the mean and standard error of 12 rings.

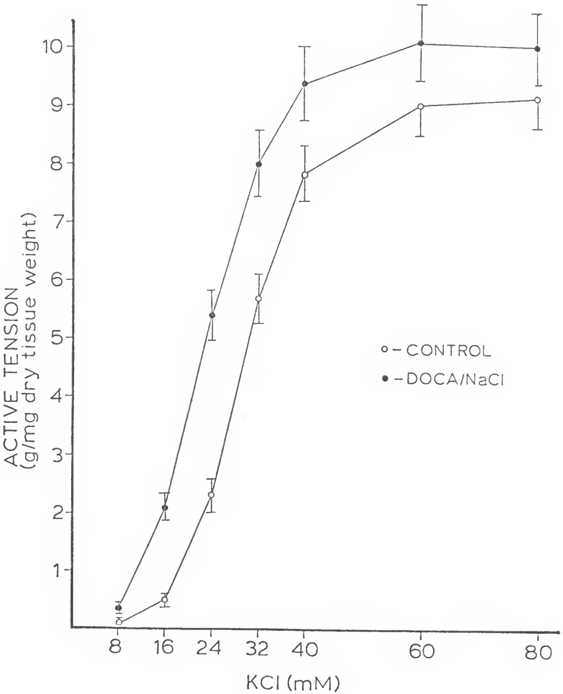

ACTIVE TENSION
(g/mg dry tissue weight)
10
9
8
7
6
5
4
3
2
1
8
16
24
32
40
60
80
KCl (mM)
○ – CONTROL
● – DOCA/NaCl

Figure 6. Dose-response relationship between concentration of KCl and percent of maximal KCl response of rings of femoral artery from control and DOCA/NaCl hypertensive rats. Each response is calculated from the data in Figure 5 and is shown as a percent of the maximum active tension generated. Vascular smooth muscle from DOCA/NaCl hypertensive rats showed an enhanced response ($p < .05$ to $p < .001$) to KCl (8 to 40 mM) when compared to controls. Each point represents the mean and standard error of 12 rings.

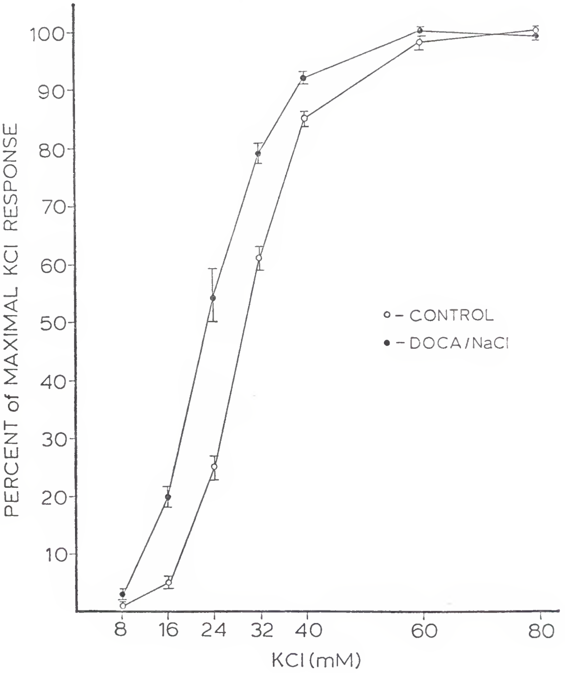
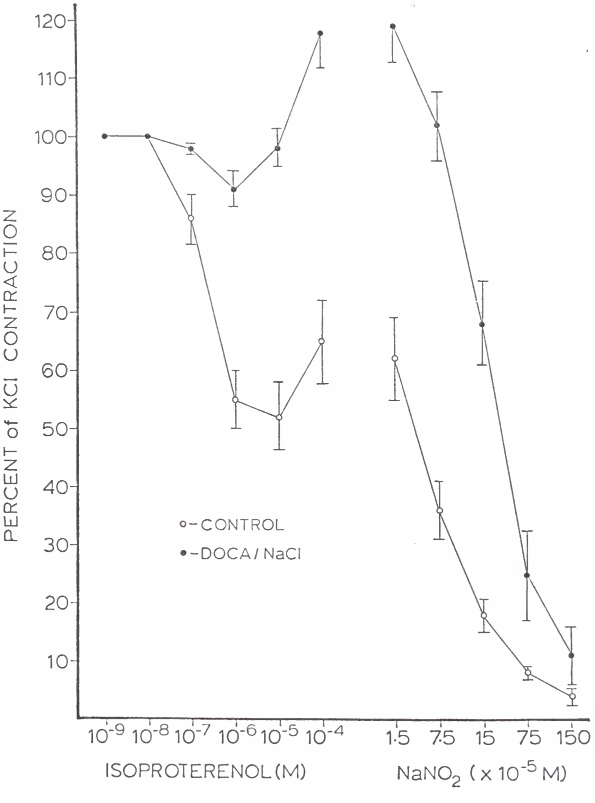

PERCENT of MAXIMAL KCl RESPONSE
100
90
80
70
60
50
40
30
20
10
8
16
24
32
40
60
80
KCl (mM)
○ – CONTROL
● – DOCA / NaCl

Figure 7. Dose-response relationship between concentration of isoproterenol and sodium nitrite and relaxation, expressed as a percent of KCl contraction, of rings of femoral artery from control and DOCA/NaCl hypertensive rats. Maximum isoproterenol-induced relaxation was significantly attenuated ($p < .001$) in vascular smooth muscle from DOCA/NaCl hypertensive rats. No difference in the maximum response to sodium nitrite was observed. Each point represents the mean and standard error of 12 rings.


PERCENT of KCl CONTRACTION
120
110
100
90
80
70
60
50
40
30
20
10
○ - CONTROL
● - DOCA / NaCl
10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5} 10^{-4}
ISOPROTERENOL (M)
1·5 7·5 15 75 150
$NaNO_2$ (x 10^{-5} M)

in tension was observed also in control rats at 10^{-4} M ISO; however, the maximal response was greater in rings of femoral artery from the DOCA/NaCl group when compared to controls (28 percent vs. 13 percent increase in tension). Relaxation in response to $NaNO_2$ was significantly different between the two groups at low concentrations; however, the maximal response was similar (96 percent relaxation in controls vs. 89 percent relaxation in DOCA/NaCl).

A significant increase in both sensitivity and contractility of femoral smooth muscle from DOCA/NaCl hypertensive rats to NE was observed when compared to controls (Figure 8). Normalizing the data and expressing it as a percent of the maximal NE response reveals that the increase in NE sensitivity (10^{-10} to 10^{-9} M) of femoral smooth muscle from the DOCA/NaCl rat remains (Figure 9). The ED10 values for control and DOCA/NaCl hypertensive rats were $1.3 \times 10^{-8} \pm 2.1 \times 10^{-9}$ M and $6.4 \times 10^{-9} \pm 1.5 \times 10^{-9}$ M, respectively.

Part B

Dose-response curves to KCl in the presence and absence of phentolamine (10^{-5} M) are shown in Figure 10. Phentolamine had no effect on the response of femoral smooth muscle from control animals. However, the maximum contractile response in the presence of phentolamine was decreased (although not significantly) in rings of femoral artery from DOCA/NaCl hypertensive rats. Figure 11 shows

Figure 8. Dose-response relationship between concentration of norepinephrine and development of active tension by rings of femoral artery from control and DOCA/NaCl hypertensive rats. Norepinephrine-induced contractions were significantly greater ($p < .05$ to $p < .001$) in vascular smooth muscle from DOCA/NaCl hypertensive rats at all concentrations. Each point represents the mean and standard error of 12 rings.

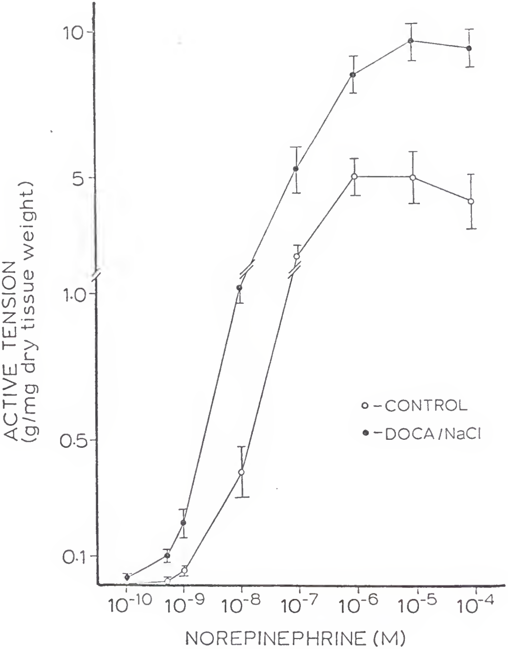

10
5
1.0
0.5
0.1
ACTIVE TENSION
(g/mg dry tissue weight)
○ - CONTROL
● - DOCA / NaCl
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
NOREPINEPHRINE (M)

Figure 9. Dose-response relationship between concentration of norepinephrine and percent of maximal norepinephrine response of rings of femoral artery from control and DOCA/NaCl hypertensive rats. Each response is calculated from the data in Figure 8 and is shown as a percent of the maximum active tension generated. A significant increase in the sensitivity ($p < .01$) to norepinephrine (10^{-10} to 10^{-9} M) was observed in vascular smooth muscle from DOCA/NaCl hypertensive rats. Each point represents the mean and standard error of 12 rings.

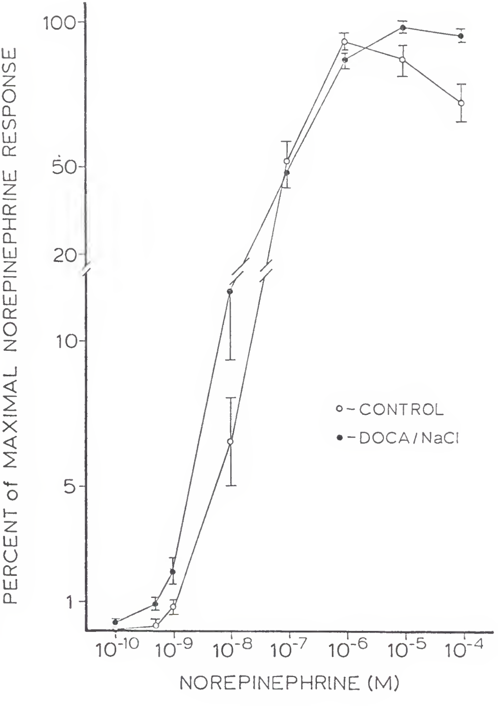

PERCENT of MAXIMAL NOREPINEPHRINE RESPONSE
100
50
20
10
5
1
10^-10
10^-9
10^-8
10^-7
10^-6
10^-5
10^-4
NOREPINEPHRINE (M)
○ - CONTROL
● - DOCA / NaCl

Figure 10. Dose-response relationship between concentration of KCl and development of active tension by rings of femoral artery from control and DOCA/NaCl hypertensive rats in the presence or absence of phentolamine (10^{-5} M). Phentolamine had no effect on vascular smooth muscle from either control or DOCA/NaCl hypertensive rats. However, femoral artery from DOCA/NaCl hypertensive rats showed a significant increase ($p < .001$) in the response to KCl (8 and 16 mM) when compared to controls. Each point represents the mean and standard error of 12 rings.

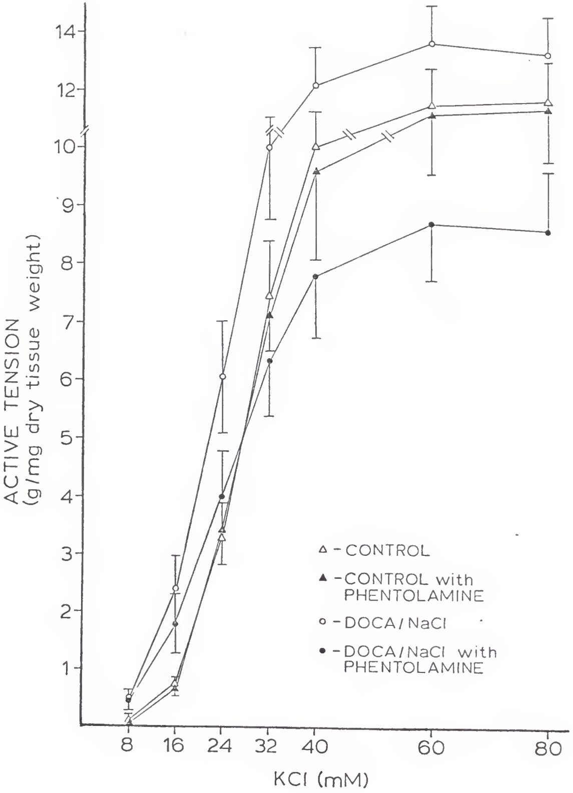
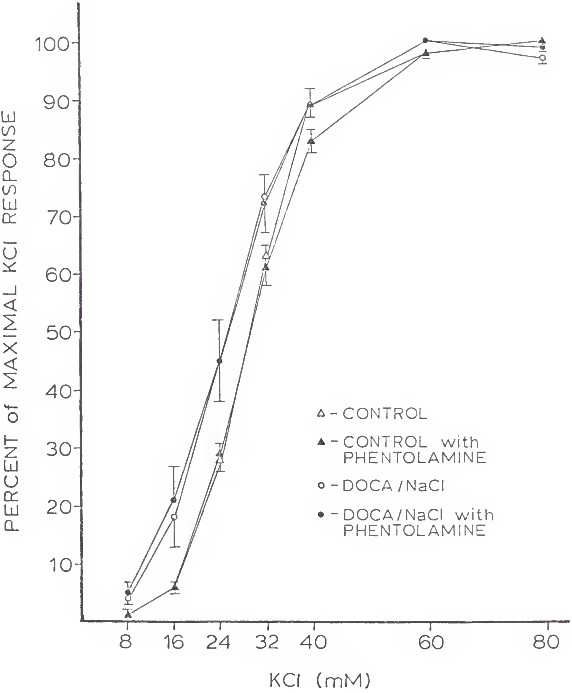

ACTIVE TENSION
(g/mg dry tissue weight)
14
12
10
9
8
7
6
5
4
3
2
1
8
16
24
32
40
60
80
KCl (mM)
△ - CONTROL
▲ - CONTROL with PHENTOLAMINE
○ - DOCA / NaCl
● - DOCA / NaCl with PHENTOLAMINE

Figure 11. Dose-response relationship between concentration of KCl and percent of maximal KCl response of rings of femoral artery from control and DOCA/NaCl hypertensive rats in the presence or absence of phentolamine (10^{-5} M). Each response is calculated from the data in Figure 10 and is shown as a percent of the maximum active tension generated. No effect of phentolamine was observed in either group while vascular smooth muscle from DOCA/NaCl hypertensive rats showed an enhanced response ($p < .05$ to $p < .001$) to KCl (8 to 32 mM) when compared to controls. Each point represents the mean and standard error of 12 rings.


PERCENT of MAXIMAL KCl RESPONSE
100
90
80
70
60
50
40
30
20
10
8
16
24
32
40
60
80
KCl (mM)
△ - CONTROL
▲ - CONTROL with PHENTOLAMINE
○ - DOCA / NaCl
● - DOCA / NaCl with PHENTOLAMINE

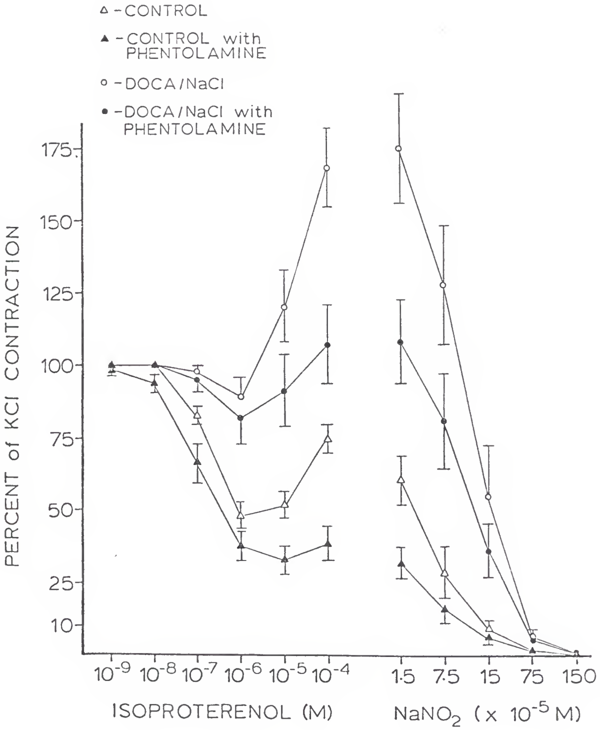
the data expressed as a percent of the maximal KCl response. Phentolamine had no effect on either control or DOCA/NaCl treated rats, but a significant difference was observed between rings of femoral artery from control and DOCA/NaCl rats at KCl concentrations of 8 to 32 mM, as was seen in Experiment II, Part A.

ISO and $NaNO_2$-induced relaxation in the presence or absence of phentolamine are shown in Figure 12. Although a slight increase in the sensitivity of femoral smooth muscle from both control and DOCA/NaCl hypertensive rats to ISO (10^{-9} to 10^{-6} M) occurred in the presence of phentolamine, the effect was not significant. Phentolamine, however, did prevent the increase in tension observed at high doses of ISO to some degree. Even though this response was partially blocked, femoral smooth muscle from the DOCA/NaCl group did respond more than controls. In addition, maximal ISO-induced relaxation of femoral smooth muscle from DOCA/NaCl rats in the absence of phentolamine was significantly attenuated, as was seen in Experiment II, Part A. No difference in the maximal response to $NaNO_2$ was observed between groups and phentolamine had no effect. Differences at the low doses of $NaNO_2$ are a result of the differences seen at 10^{-4} M ISO.

Discussion

The experiments in this section were designed to investigate alterations in vascular reactivity of rings of

Figure 12. Dose-response relationship between concentration of isoproterenol and sodium nitrite and relaxation, expressed as a percent of KCl contraction, of rings of femoral artery from control and DOCA/NaCl hypertensive rats in the presence or absence of phentolamine (10^{-5} M). A significant decrease ($p < .001$) in the maximal response to isoproterenol was observed in vascular smooth muscle from DOCA/NaCl hypertensive rats. In addition, phentolamine had a significant effect ($p < .05$) on rings of femoral artery from both control and DOCA/NaCl hypertensive rats in preventing the contraction induced by high concentrations of isoproterenol (10^{-5} to 10^{-4} M). Each point represents the mean and standard error of 12 rings.


△ - CONTROL
▲ - CONTROL with PHENTOLAMINE
○ - DOCA / NaCl
● - DOCA / NaCl with PHENTOLAMINE
PERCENT of KCl CONTRACTION
175
150
125
100
75
50
25
10
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
1·5
7·5
15
75
150
ISOPROTERENOL (M)
$NaNO_2$ ($\times 10^{-5}$ M)

femoral artery from DOCA/NaCl hypertensive rats. Potassium chloride (KCl) a nonspecific smooth muscle stimulant (depolarizes the smooth muscle membrane) was used to test the basic contractile machinery of the VSM. Isoproterenol (ISO) and norepinephrine (NE) were used to test β- and α-adrenergic responsiveness, respectively, of the VSM. Sodium nitrite ($NaNO_2$), a nonspecific smooth muscle relaxant, was used to investigate the innate ability of the VSM to relax.

The responsiveness of VSM form DOCA/NaCl hypertensive rats to KCl was significantly greater than controls. This increase in reactivity may be due to several factors. Since KCl-induced contraction is dependent upon an influx of extracellular calcium ion (Ca^{++})(Hudgins and Weiss, 1968), it is possible that the increase in responsiveness of VSM from the DOCA/NaCl rat is due to an increase in the permeability of the membrane to Ca^{++}.

Field _et_ _al_. (1972) and Fitzpatrick and Szentivanyi (1980) have observed an increase in the response of aortic smooth muscle from SHR to low concentrations of KCl. Similar results were obtained by Cheng and Shibata (1980) using a perfused hindquarter preparation of the SHR. On the other hand, Mulvany and Nyborg (1980) saw no difference in the responsiveness of mesenteric resistance vessels from SHR and WKY to KCl.

An increase in the permeability of femoral smooth muscle from DOCA/NaCl hypertensive rats to Ca^{++} has been suggested by Holloway and Bohr (1973), since dose-response curves to KCl were significantly greater at concentrations of 15 to 45 mM in the DOCA/NaCl group. Hansen and Bohr (1975), however, observed increased in the responsiveness of femoral strips from DOCA/NaCl hypertensive rats to low concentrations of KCl only.

Another possible reason for the increased responsiveness of VSM from DOCA/NaCl hypertensive rats may be due to the release of NE from adrenergic nerve terminals. Lorenz and Vanhoutte (1975) have shown that depolarizing levels of potassium can release NE from noradrenergic nerve terminals in isolated vascular segments which results in vasoconstriction. Since the femoral artery has some innveration, it is possible that the increased response may be due, in part, to the release of NE. Potassium chloride dose-response curves were generated in the presence of phentolamine. Phentolamine had no effect on rings of femoral artery from control animals; however, the response of VSM from DOCA/NaCl rats was decreased at high concentrations of KCl. The responses to low concentrations of KCl were still significantly greater in the DOCA/NaCl group when compared to controls. This suggests that the increase in responsiveness to KCl at low doses is due to an increase in Ca^{++} permeability or sensitivity, while the increase in contractility of the VSM is due, in part, to NE release and activation of α-adrenergic receptors.

A third possible cause for the increase in responsiveness may be a result of the VSM cell hyperplasia as described in Experiment I. The increased number of smooth muscle cells provides more contractile machinery to be activated and therefore produces a greater response. Since VSM from DOCA/NaCl hypertensive rats generated a greater maximal active tension, the differences at low concentrations of KCl may simply be a reflection of more VSM. To account for these differences, the responses reported as active tension were normalized and expressed as a percent of the maximal contraction. As can be seen in Figure 6 the differences in VSM responsiveness to KCl were still evident. Therefore, it appears that the increase in vascular reactivity of DOCA/NaCl hypertensive rats to KCl may be due to an increase in the Ca^{++} permeability or sensitivity of the VSM.

Another index of a possible alteration in Ca^{++} handling in VSM from DOCA/NaCl hypertensive rats is seen in the $t_{\frac{1}{2}}$ value for the KCl washout curve. Wei et al. (1976) have shown a decrease in Ca^{++} pump activity (ATP-dependent) in VSM of SHR, while Field et al. (1972) have shown that aortic rings from SHR relax slower than controls after removal of a potassium-depolarizing physiological solution. The increased $t_{\frac{1}{2}}$ for the KCl washout curve of rings of femoral artery from DOCA/NaCl hypertensive rats may be due to a decreased ability of the VSM cell to sequester Ca^{++}. It is not known if this decrease in Ca^{++} pump activity contributes to the increase in VSM responsiveness to KCL. The issue of an increase in Ca^{++} permeability causing the increased responsiveness to KCl in VSM from DOCA/NaCl hypertensive rats will be dealt with in Experiment III.

Beta-adrenergic mediated responsiveness was investigated in VSM from control and DOCA/NaCl hypertensive rats by measuring ISO-induced relaxation of rings of femoral artery precontracted with KCl. A significant reduction in maximal relaxation was observed in the DOCA/NaCl group. Similar results have been obtained by Katovich et al. (1984).

This attenuated response could be due to several factors, one of which involves alterations in the β-receptor itself. A decrease in the affinity or number of receptors for ISO could possibly explain the decrease in response. Using radioligand binding studies, reduced numbers of β-receptors have been reported in the aorta of SHR (Limas and Limas, 1979), as well as mesenteric artery from DOCA/NaCl hypertensive rats (Woodcock et al., 1980). No change in the affinity of the receptor was seen.

Beta-receptor stimulation results in an increase in the intracellular levels of cyclic adenosine 3',5'-monophosphate (cyclic AMP) which has been hypothesized to be the mediator for relaxation in VSM (Triner et al., 1971; Anderson, 1973). A decrease in the relaxation response of VSM from SHR to exogenous application of cyclic AMP has been observed (Cohen and Berkowitz, 1976). In addition, ISO-induced cyclic AMP accumulation is decreased in thoracic aorta from SHR (Schoeffter and Stoclet, 1983). Similar studies, however, have not been done in the DOCA/NaCl hypertensive rat to date.

Cyclic AMP is known to activate a protein kinase which is thought to catalyze a protein phosphorylation leading to a reduction in Ca^{++} and thus causing a relaxation of VSM (Robison et al., 1971). This reduction in Ca^{++} may occur through a direct mechanism by way of activation of a Ca^{++}-ATPase (Ca^{++} pump) in the plasma membrane and/or sacroplasmic reticulum (Mueller and van Breemen, 1979; Kroeger et al., 1975; van Breemen, 1977), or indirectly by stimulation of the membrane Na,K-ATPase (sodium-potassium pump) (Scheid et al., 1979). It is possible, therefore, that the decrease in β-receptor mediated relaxation in VSM from DOCA/NaCl hypertensive rats may be due to an alteration in any one of these mechanisms. Possible alterations in Ca^{++} pump activity have already been discussed in terms of the KCl washout curves. Alterations in the sodium-potassium pump are known to occur in the DOCA/NaCl hypertensive rat, however, its role in alterations in β-receptor mediated relaxation is not known at this point in time. This issue will be dealt with in Part V of this dissertation.

Responsiveness of VSM from DOCA/NaCl hypertensive rats to KCl was shown in this experiment, and appears to be due to an increased Ca^{++} permeability. Since KCl was used as the contractile agent for the ISO dose-response curve, it may be possible that the increased permeability of VSM from DOCA/NaCl rats to Ca^{++} in response to KCl offsets, to some degree, the cyclic AMP-mediated Ca^{++} sequestration induced by ISO. The possible role for this increase in Ca^{++} permeability resulting in a decrease in the ISO-induced relaxation of VSM from DOCA-NaCl hypertensive rats will be addressed in Experiment III.

Finally, the question arises as to whether the reduced β-adrenergic response is actually a receptor phenomenon or simply an alteration in the ability of the VSM to relax. To answer this, $NaNO_2$, a nonspecific smooth muscle relaxing agent, was used to test the ability of the VSM to relax. All tissues were able to relax, indicating that the reduction in the ISO-induced relaxation is indeed a receptor-mediated phenomenon. Therefore, this supports the observations of Woodcock et al. (1980) that a decrease in the number of vascular β-adrenergic receptors occurs in the DOCA/NaCl hypertensive rat which results in the decrease in ISO-induced relaxation.

At higher concentrations of ISO (10^{-5} to 10^{-4} M) an increase in tension was observed. This is believed to be due to an α-adrenergic receptor stimulating component of ISO. Although ISO is predominantly a β-adrenergic agonist, it has been shown to possess α-adrenergic agonistic properties (Furchgott, 1954; Flaim and Zelis, 1977). It was observed that in VSM from the DOCA/NaCl hypertensive rat the increase in tension to ISO was greater than controls.

This in itself could reflect an enhanced α-adrenergic component of VSM from the DOCA/NaCl rat.

Since there appears to be an enhanced α-adrenergic responsiveness in the DOCA/NaCl hypertensive rat as demonstrated by the high concentrations of ISO, it may be possible that the decrease in β-adrenergic responsiveness is due, in part, to an enhanced α-adrenergic vasoconstrictor tone. Therefore, ISO dose-response curves were carried out in the presence of phentolamine to block the α-adrenergic component. In VSM from both control and DOCA/NaCl hypertensive rats, phentolamine had a slight enhancing effect on ISO-induced relaxation while having a significant attenuating effect on ISO-induced contraction.

It has been reported that phentolamine at high enough doses can have nonspecific effects on VSM contraction and relaxation (Hof and Vuorela, 1983). In light of the present studies and their results, it is suggested by this author that phentolamine not be used in delineating the α- and β-adrenergic components of ISO in VSM preparations. Since the maximal response (relaxation) to ISO was not altered, it would appear fruitless to use phentolamine in these studies. In addition, the contractile response to ISO lends further information to the alterations that occur in α-adrenergic responsiveness of VSM from DOCA/NaCl hypertensive rats.

Alpha-adrenergic responsiveness to NE in rings of femoral artery from DOCA/NaCl hypertensive rats was significantly greater than controls. An increase in both the sensitivity and contractility of VSM to NE stimulation was observed. The increase in sensitivity has been reported also in rings of aortic smooth muscle (Katovich *et al*., 1984), isolated perfused kidneys (Berecek *et al*., 1980), and femoral artery strips (Hansen and Bohr, 1975) from the DOCA/NaCl hypertensive rat. However, the maximal contractility in response to NE has varied. While an increase was observed in this dissertation and in the work by Katovich *et al*. (1984), others have shown a decrease (Hansen and Bohr, 1975; Pang and Sutter, 1980). This difference is most likely explained by the alterations in vessel morphology that occur during the hypertensive process. Many studies on *in vitro* VSM responsiveness have been done on animals with well-established hypertension. The vasculature of these animals has been exposed to high pressures for an extended period of time and, therefore, structural alterations such as increases in collagen and elastin have occurred. These changes result in a decrease in the maximal contractility of the VSM. However, the vasculature of animals that has been exposed to the increase in pressure for only a few weeks exhibit structural alterations that involve only a hyperplasia of VSM cells. This would contribute to an increase in the contractility as well as sensitivity of the VSM to NE. Measurements of smooth muscle cells, and collagen and elastin have shown this to be the case (Cox, 1982; Mangiarua *et al*., 1981).

In addition, Katovich et al. (1984) have shown that the sensitivity and maximal contractility of aortic rings from DOCA/NaCl hypertensive rats is greater in the early stages of hypertension, whereas, maximal contractility is decreased in the established phase of hypertension.

Therefore, the increase in NE sensitivity, as well as contractility observed in VSM from DOCA/NaCl hypertensive rat in this study may be due, in part, to the increase in smooth muscle mass.

Since the increase in NE sensitivity may not be a true increase, but merely a reflection of the increased contractility, the data are normalized and expressed as a percent of the maximal NE response. As can be seen in Figure 9, the increase in sensitivity of VSM from DOCA/NaCl hypertensive rats to low doses of NE still exists. Also, the increased sensitivity is further shown by the lower ED10 values in the DOCA/NaCl group.

Although no studies have been done using radioligand binding to α-adrenergic receptors in VSM of hypertensive animals, one of the possibilities for the increase in responsiveness to NE may be an increase in the number and/or affinity of the α-adrenergic receptor for NE.

Another possible reason for the increase in NE responsiveness may be due to an increase in the permeability of the VSM to Ca^{++}. Several investigators have observed an increase in Ca^{++} sensitivity of VSM from SHR (Mulvany et al., 1981; Aoki _et al_., 1980; Fitzpatrick and Szentivanyi, 1980) and suggest that the increase in NE responsiveness is due to an increase in the permeability of the VSM to Ca^{++}. Few studies, however, have been done on the DOCA/NaCl hypertensive rat in terms of the role of Ca^{++} in the altered response to NE. Therefore, the third experiment in this dissertation will address this issue in more detail.

Whatever the cause for the increase in NE sensitivity, it is widely accepted that the increase does occur in the DOCA/NaCl hypertensive rat. It also appears that the increase in sensitivity may be a primary causative factor for the increase in peripheral vascular resistance since time course studies have shown that an increase in VSM reactivity to NE occurs prior to the onset of hypertension (Katovich _et al_., 1984; Berecek _et al_., 1980).

Experiment III

This experiment was designed to determine the role of extracellular calcium ion in the alterations that occur in vascular reactivity of the DOCA/NaCl hypertensive rat.

Methods

Twenty-four 6 week-old male S-D rats were used in this experiment. DOCA/NaCl hypertension was induced in 12 of these rats as described in General Methods. At 10 weeks of age (4 weeks postimplant) all animals were sacrificed and rings of femoral artery prepared. The 90 minute equilibration period and dose-response curves to KCl, ISO,

$NaNO_2$, and NE were carried out in either a low (0.25 mM) or high (7.5 mM) calcium modified Krebs physiological solution at the appropriate preload force.

Results

Development of active tension by rings of femoral artery from DOCA/NaCl hypertensive rats in response to accumulative addition of KCl in the high calcium Krebs was significantly greater than controls at concentrations of 8 to 32 mM (Figure 13). Expressing the data as a percent of the maximal KCl response (Figure 14) reveals an enhanced responsiveness of femoral smooth muscle from the DOCA/NaCl group to KCl at all concentrations, except 80 mM. The $t_{\frac{1}{2}}$ values for KCl washout were 124 ± 20 seconds and 28 ± 3 seconds for DOCA/NaCl and controls, respectively ($p < .001$).

Isoproterenol-induced relaxation in high calcium Krebs was significantly attenuated in femoral smooth muscle from DOCA/NaCl hypertensive rats (Figure 15). The initial and maximal response (11 percent relaxation) to ISO occurred at 10^{-6} M in DOCA/NaCl treated rats. Femoral smooth muscle from control rats responded initially at 10^{-7} M ISO (8 percent relaxation) with maximal relaxation occurring at 10^{-6} M (30 percent relaxation). An increase in tension was observed in both groups at 10^{-5} and 10^{-4} M ISO with the maximal response being greater in the DOCA/NaCl group (58 percent vs. 19 percent). Maximal relaxation in response to accumulative addition of $NaNO_2$ was similar in

Figure 13. Dose-response relationship between concentration of KCl and development of active tension by rings of femoral artery from control and DOCA/NaCl hypertensive rats equilibrated in a high calcium (7.5 mM) Krebs physiological solution. The response of vascular smooth muscle from DOCA/NaCl hypertensive rats to KCl (8 to 32 mM) was significantly greater ($p < .05$ to $p < .01$) than controls. Each point represents the mean and standard error of 12 rings.

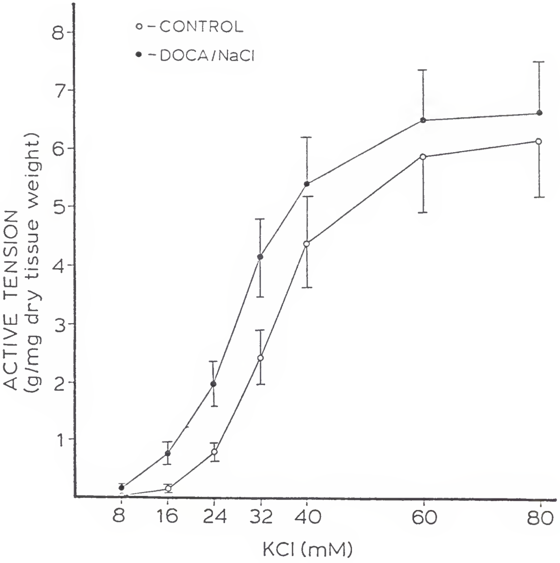

○ - CONTROL
● - DOCA/NaCl
ACTIVE TENSION
(g/mg dry tissue weight)
8
7
6
5
4
3
2
1
8
16
24
32
40
60
80
KCl (mM)

Figure 14. Dose-response relationship between concentration of KCl and percent of maximal KCl response of rings of femoral artery from control and DOCA/NaCl hypertensive rats equilibrated in a high calcium (7.5 mM) Krebs physiological solution. Each response is calculated from the data in Figure 13 and is shown as a percent of the maximum active tension generated. Vascular smooth muscle from DOCA/NaCl hypertensive rats shown an enhanced response ($p < .05$ to $p < .001$) to KCl (8 to 60 mM) when compared to controls. Each point represents the mean and standard error of 12 rings.

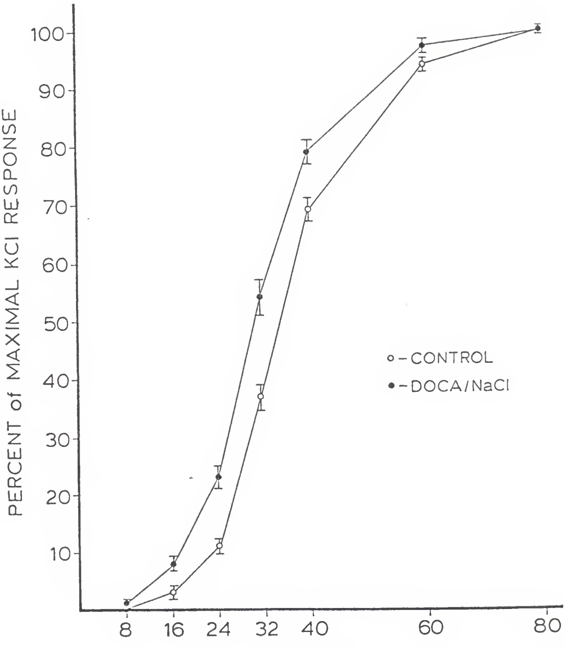
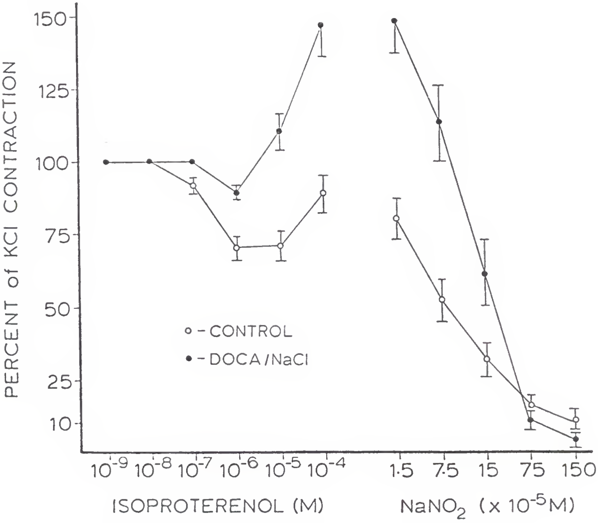

PERCENT of MAXIMAL KCl RESPONSE
100
90
80
70
60
50
40
30
20
10
8
16
24
32
40
60
80
KCl (mM)
○ - CONTROL
● - DOCA/NaCl

Figure 15. Dose-response relationship between concentration of isoproterenol and sodium nitrite and relaxation, expressed as a percent of KCl contraction, of rings of femoral artery from control and DOCA/NaCl hypertensive rats equilibrated in a high calcium (7.5 mM) Krebs physiological solution. Maximum isoproterenol-induced relaxation was significantly attenuated ($p < .001$) in vascular smooth muscle from DOCA/NaCl hypertensive rats. No difference in the maximum response to sodium nitrite was observed. Each point represents the mean and standard error of 12 rings.


PERCENT of KCl CONTRACTION
150
125
100
75
50
25
10
○ - CONTROL
● - DOCA/NaCl
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
ISOPROTERENOL (M)
1·5
7·5
15
75
150
$NaNO_2$ ($\times 10^{-5}$M)

both groups (96 percent relaxation in DOCA/NaCl vs. 89 percent relaxation in controls).

Femoral smooth muscle from the DOCA/NaCl hypertensive rat showed a significant increase in both sensitivity and contractility to NE stimulation in the high calcium Krebs (Figure 16). A significant increase in NE sensitivity and contractility was evident also when the data are expressed as a percent of the maximal response to NE (Figure 17). ED10 values for control and DOCA/NaCl hypertensive rats were $1.4 \times 10^{-8} \pm 2.0 \times 10^{-9}$ M and $6.2 \times 10^{-9} \pm 1.4 \times 10^{-9}$ M, respectively (Table 2).

Dose-response curves of femoral smooth muscle from control and DOCA/NaCl hypertensive rats to KCl in the low calcium Krebs solution are shown in Figure 18. Rings of femoral artery from DOCA/NaCl treated rats showed an enhanced response at 8 and 16 mM KCl when compared to controls, however, maximum contractility was significantly decreased (2.4 vs. 5.2 g/mg dry tissue weight). When the data are expressed as a percent of the maximal KCl response, femoral smooth muscle from the DOCA/NaCl hypertensive rat showed a highly significant increase in the response to KCl at 8 to 40 mM (Figure 19). The $t_{\frac{1}{2}}$ values for KCl washout were 139 ± 36 seconds and 28 ± 1 second for DOCA/NaCl and controls, respectively ($p < .001$).

Rings of femoral artery from both groups showed an increase in the sensitivity to ISO stimulation in the low calcium Krebs with initial responses occurring at 10^{-9} M

Figure 16. Dose-response relationship between concentration of norepinephrine and development of active tension by rings of femoral artery from control and DOCA/NaCl hypertensive rats equilibrated in a high calcium (7.5 mM) Krebs physiological solution. Norepinephrine-induced contractions were significantly greater ($p < .05$ to $p < .001$) in vascular smooth muscle from DOCA/NaCl hypertensive rats at all concentrations. Each point represents the mean and standard error of 12 rings.

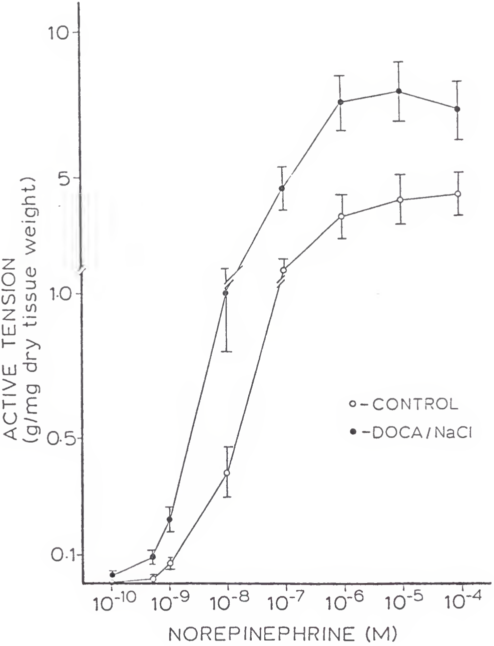

10
5
1.0
0.5
0.1
ACTIVE TENSION
(g/mg dry tissue weight)
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
NOREPINEPHRINE (M)
○ - CONTROL
● - DOCA / NaCl

Figure 17. Dose-response relationship between concentration of norepinephrine and percent of maximal norepinephrine response of rings of femoral artery from control and DOCA/NaCl hypertensive rats equilibrated in a high calcium (7.5 mM) Krebs physiological solution. Each response is calculated from the data in Figure 16 and is shown as a percent of the maximum active tension generated. Norepinephrine-induced contractions were significantly greater ($p < .05$) in vascular smooth muscle from DOCA/NaCl hypertensive rats at all concentrations. Each point represents the mean and standard error of 12 rings.

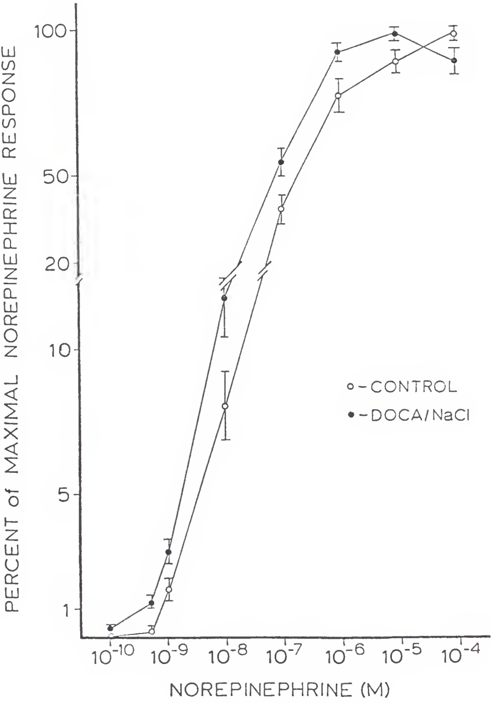

PERCENT of MAXIMAL NOREPINEPHRINE RESPONSE
100
50
20
10
5
1
10^-10
10^-9
10^-8
10^-7
10^-6
10^-5
10^-4
NOREPINEPHRINE (M)
○ - CONTROL
● - DOCA/NaCl

Figure 18. Dose-response relationship between concentration of KCl and development of active tension by rings of femoral artery from control and DOCA/NaCl hypertensive rats equilibrated in a low calcium (0.25 mM) Krebs physiological solution. The response of vascular smooth muscle from DOCA/NaCl hypertensive rats to KCl (8 and 16 mM) was significantly greater ($p < .01$) than controls, however, maximal contraction (60 to 80 mM) was significantly reduced ($p < .001$) in the DOCA/NaCl group. Each point represents the mean and standard error of 12 rings.

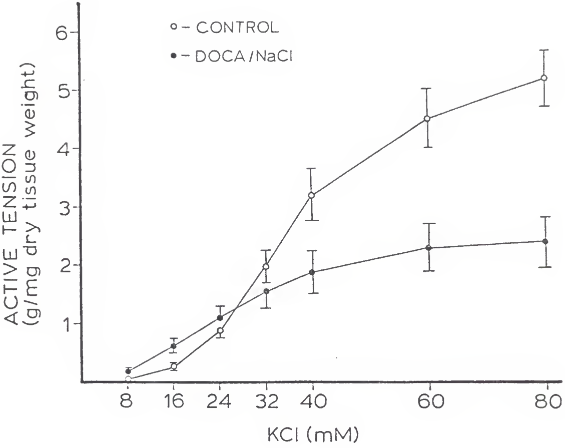
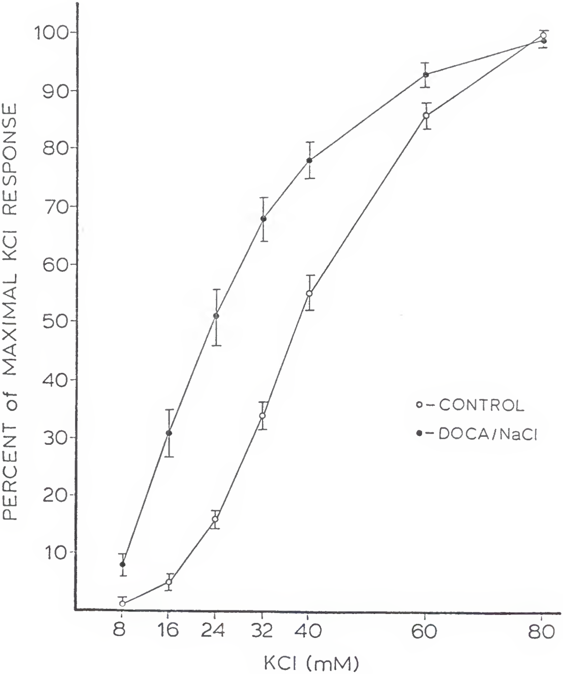

○ – CONTROL
● – DOCA / NaCl
ACTIVE TENSION
(g/mg dry tissue weight)
1
2
3
4
5
6
8
16
24
32
40
60
80
KCl (mM)

Figure 19. Dose-response relationship between concentration of KCl and percent of maximal KCl response of rings of femoral artery from control and DOCA/NaCl hypertensive rats equilibrated in a low calcium (0.25 mM) Krebs physiological solution. Each response is calculated from the data in Figure 18 and is shown as a percent of the maximum active tension generated. Vascular smooth muscle from DOCA/NaCl hypertensive rats showed an enhanced response ($p < .001$) to KCl (8 to 40 mM) when compared to controls. Each point represents the mean and standard error of 12 rings.


PERCENT of MAXIMAL KCl RESPONSE
100
90
80
70
60
50
40
30
20
10
8
16
24
32
40
60
80
KCl (mM)
○ – CONTROL
● – DOCA/NaCl

(Figure 20)(5 percent relaxation in DOCA/NaCl vs. 3 percent relaxation in controls). Maximal ISO-induced relaxation in the DOCA/NaCl treated rat occurred at 10^{-7} M (11 percent relaxation), however, it remained significantly attenuated when compared to controls (49 percent relaxation). An increase in tension occurred at 10^{-5} and 10^{-4} M ISO, as was seen previously in both normal and high calcium Krebs (21 percent in DOCA/NaCl vs. 13 percent in controls). Again, maximal relaxation in response to $NaNO_2$ was similar for both groups (100 percent in DOCA/NaCl vs. 99 percent in controls). Differences at low doses of $NaNO_2$ are a result of the differences at 10^{-4} M ISO.

No differences in the response to accumulative addition of NE were observed between control and DOCA/NaCl hypertensive rats in the low calcium Krebs (Figures 21 and 22). ED10 values for control and DOCA/NaCl hypertensive rats were $1.25 \times 10^{-8} \pm 2.3 \times 10^{-9}$ M and $8.6 \times 10^{-9} \pm 2.1 \times 10^{-9}$ M, respectively (Table 2).

To more clearly show the effects of altered Ca^{++} on VSM reactivity to KCl, responses at each concentration of Ca^{++} were graphed together. Figure 23 shows the responses of rings of femoral artery from controls at 2.5 mM, 0.25 mM, and 7.5 mM Ca^{++}. A decrease in maximal active tension of approximately 40 percent was observed in the low Ca^{++} Krebs, while a 30 percent decrease was seen in the high calcium Krebs. On the other hand, rings of femoral artery from

Figure 20. Dose-response relationship between concentration of isoproterenol and sodium nitrite and relaxation, expressed as a percent of KCl contraction, of rings of femoral artery from control and DOCA/NaCl hypertensive rats equilibrated in a low calcium (0.25 mM) Krebs physiological solution. Maximum isoproterenol-induxed relaxation was significantly attenuated ($p < .001$) in vascular smooth muscle from DOCA/NaCl hypertensive rats. No difference in the maximum response to sodium nitrite was observed. Each point represents the mean and standard error of 12 rings.

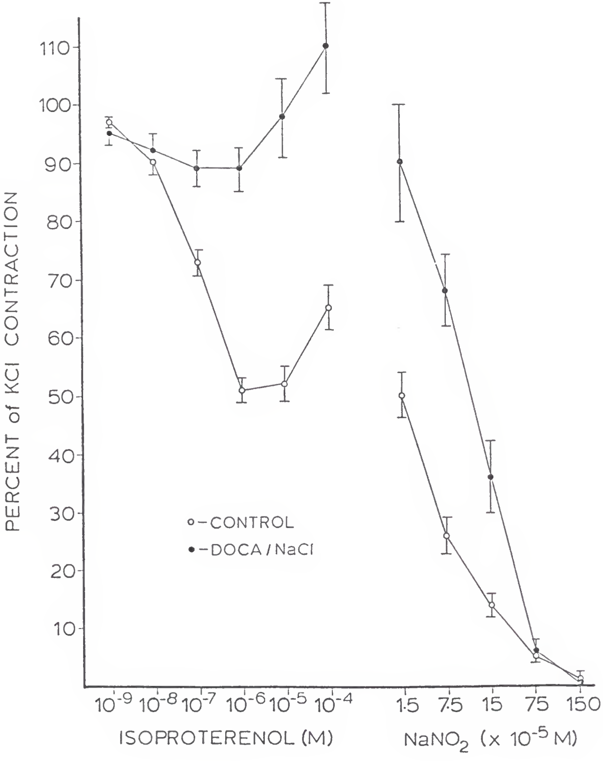

110
100
90
80
70
60
50
40
30
20
10
PERCENT of KCl CONTRACTION
○ – CONTROL
● – DOCA / NaCl
10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5} 10^{-4}
ISOPROTERENOL (M)
1·5 7·5 15 75 150
NaNO$_2$ (x 10^{-5} M)

Figure 21. Dose-response relationship between concentration of norepinephrine and development of active tension by rings of femoral artery from control and DOCA/NaCl hypertensive rats equilibrated in a low calcium (0.25 mM) Krebs physiological solution. No difference in the response of vascular smooth muscle to norepinephrine was observed between control and DOCA/NaCl hypertensive rats. Each point represents the mean and standard error of 12 rings.

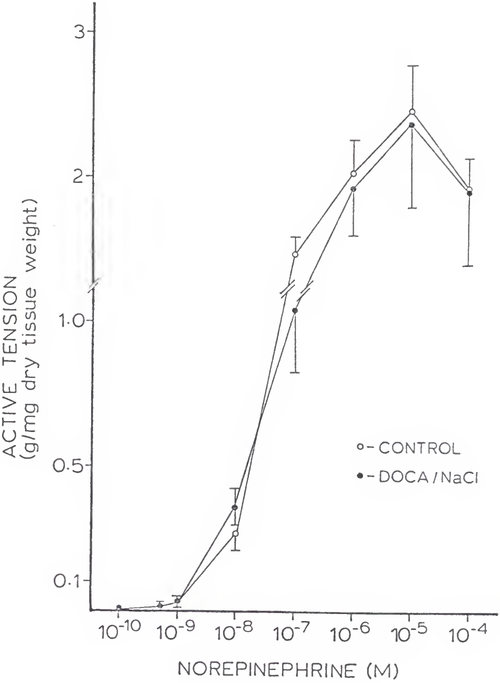

ACTIVE TENSION
(g/mg dry tissue weight)
3
2
1.0
0.5
0.1
10-10
10-9
10-8
10-7
10-6
10-5
10-4
NOREPINEPHRINE (M)
○ - CONTROL
● - DOCA / NaCl

Figure 22. Dose-response relationship between concentration of norepinephrine and percent of maximal norepinephrine response of rings of femoral artery from control and DOCA/NaCl hypertensive rats equilibrated in a low calcium (0.25 mM) Krebs physiological solution. Each response is calculated from the data in Figure 21 and is shown as a percent of the maximum active tension generated. No difference in the response of vascular smooth muscle to norepinephrine was observed between control and DOCA/NaCl hypertensive rats. Each point represents the mean and standard error of 12 rings.

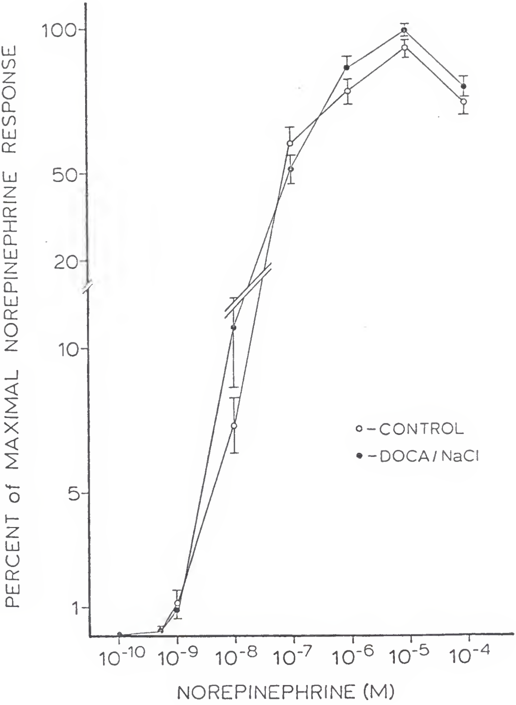
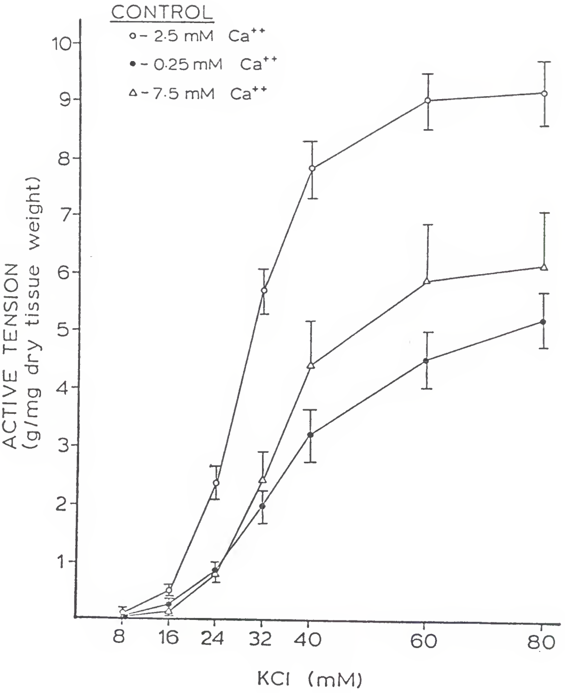

PERCENT of MAXIMAL NOREPINEPHRINE RESPONSE
100
50
20
10
5
1
10-10
10-9
10-8
10-7
10-6
10-5
10-4
NOREPINEPHRINE (M)
○ – CONTROL
● – DOCA / NaCl

Figure 23. Dose-response relationship between concentration of KCl and development of active tension by rings of femoral artery from control rats equilibrated in a low (0.25 mM), normal (2.5 mM), or high (7.5 mM) calcium Krebs physiological solution. A significant decrease ($p < .05$) in the response of vascular smooth muscle to KCl (16 to 80 mM) was observed in both low and high calcium Krebs when compared to responses in a normal calcium Krebs media. Each point represents the mean and standard error of 12 rings.


CONTROL
○ – 2·5 mM Ca^{++}
● – 0·25 mM Ca^{++}
△ – 7·5 mM Ca^{++}
ACTIVE TENSION
(g/mg dry tissue weight)
10
9
8
7
6
5
4
3
2
1
8
16
24
32
40
60
80
KCl (mM)

DOCA/NaCl hypertensive rats exhibited an 80 percent decrease in maximum active tension in low calcium Krebs and a 40 percent decrease in the high Ca^{++} Krebs (Figure 24).

Figures 25 and 26 show the KCl dose-response curve data expressed as a percent of the maximal KCl response for controls and DOCA/NaCl hypertensive rats, respectively. These figures further emphasize the differences in responsiveness of VSM to KCl at varying levels of extracellular Ca^{++}. In both controls and DOCA/NaCl treated rats the effect of high Ca^{++} was to shift the dose-response curve of KCl down and to the right. Low Ca^{++} in controls also caused a shift to the right in the dose-response curve. In VSM from the DOCA/NaCl hypertensive rat, however, the responses at 8 and 16 mM KCl in low calcium were greater than in 2.5 and 7.5 mM Ca^{++}. Although the sensitivity of VSM from DOCA/NaCl hypertensive rats to ISO was significantly increased at concentrations of 10^{-9} to 10^{-7} M in the low Ca^{++} Krebs, maximal ISO-induced relaxation was not altered (Figure 27). Whereas the initial response to ISO in normal Ca^{++} occurred at 10^{-7} M, a response in high Ca^{++} did not occur until 10^{-6} M. At concentrations of 10^{-5} and 10^{-4} M, ISO caused a contraction of VSM from DOCA/NaCl hypertensive rats at all levels of extracellular Ca^{++}. The increase in tension was, however, Ca^{++}-concentration dependent with the maximal response occurring in the high Ca^{++} Krebs. This increase in tension was discussed in

Figure 24. Dose-response relationship between concentration of KCl and development of active tension by rings of femoral artery from DOCA/NaCl hypertensive rats equilibrated in a low (0.25 mM), normal (2.5 mM), or high (7.5 mM) calcium Krebs physiological solution. Vascular smooth muscle responses to KCl (16 to 80 mM) in the low calcium media were significantly decreased ($p < .001$). Responses in the high calcium Krebs were slightly depressed ($p < .05$) when compared to responses in the normal calcium media but were still significantly greater ($p < .01$) than that in the low calcium Krebs. Each point represents the mean and standard error of 12 rings.

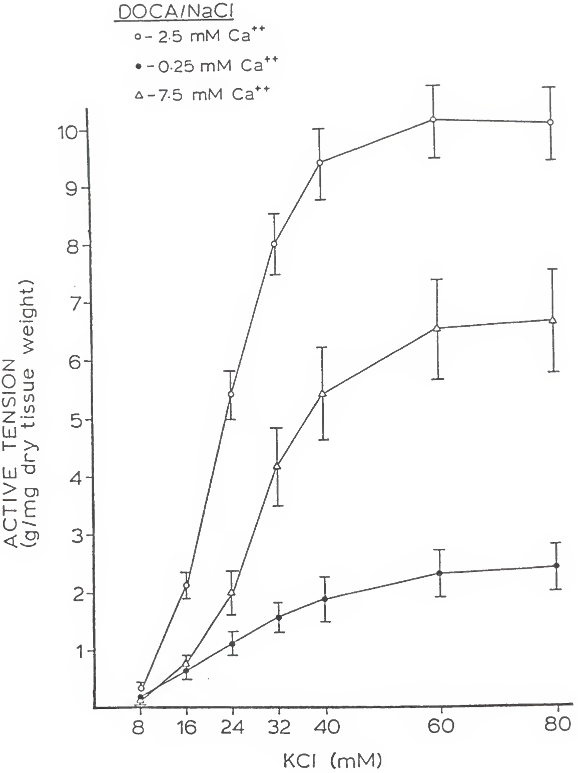

DOCA/NaCl
○ - 2·5 mM Ca⁺⁺
● - 0·25 mM Ca⁺⁺
△ - 7·5 mM Ca⁺⁺
ACTIVE TENSION
(g/mg dry tissue weight)
10
9
8
7
6
5
4
3
2
1
8
16
24
32
40
60
80
KCl (mM)

Figure 25. Dose-response relationship between concentration of KCl and percent of maximal KCl response of rings of femoral artery from control rats equilibrated in a low (0.25 mM), normal (2.5 mM), or high (7.5 mM) calcium Krebs physiological solution. Each response is calculated from the data in Figure 23 and is shown as a percent of the maximum active tension generated. A significant decrease ($p < .05$) in the response of vascular smooth muscle to KCl (24 to 60 mM) was observed in both low and high calcium Krebs when compared to responses in a normal calcium Krebs media. Responses in the low calcium Krebs were slightly less than those in the high calcium media at 40 and 60 mM KCl. Each point represents the mean and standard error of 12 rings.

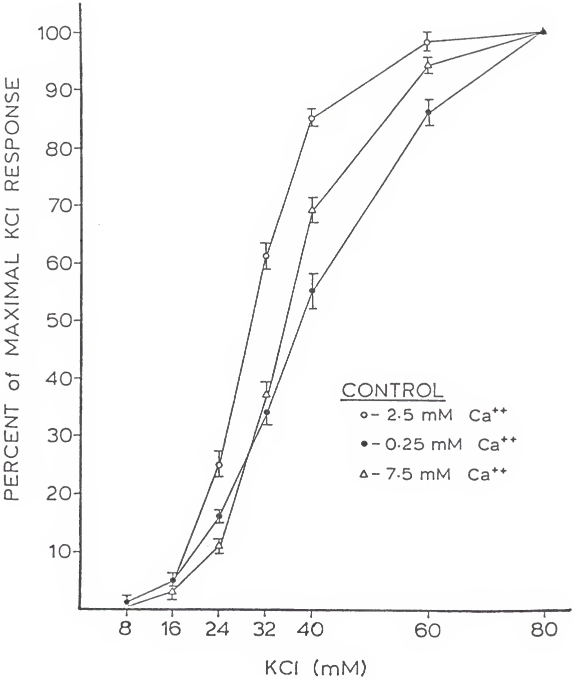

PERCENT of MAXIMAL KCl RESPONSE
100
90
80
70
60
50
40
30
20
10
8
16
24
32
40
60
80
KCl (mM)
CONTROL
○ – 2·5 mM Ca++
● – 0·25 mM Ca++
△ – 7·5 mM Ca++

Figure 26. Dose-response relationship between concentration of KCl and percent of maximal KCl response of rings of femoral artery from DOCA/NaCl hypertensive rats equilibrated in a low (0.25 mM), normal (2.5 mM), or high (7.5 mM) calcium Krebs physiological solution. Each response is calculated from the data in Figure 24 and is shown as a percent of the maximum active tension generated. Responses of vascular smooth muscle to low concentrations of KCl (8 to 24 mM) in the high calcium media were depressed ($p < .01$) when compared to responses in low and normal calcium. A significant decrease ($p < .05$) in the response to high concentrations of KCl (32 to 60 mM) was observed in both the high and low calcium Krebs solutions when compared to normal calcium. Each point represents the mean and standard error of 12 rings.

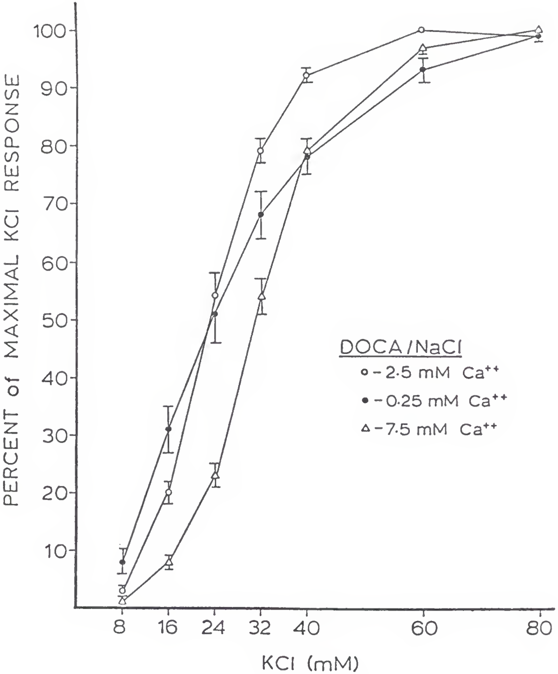
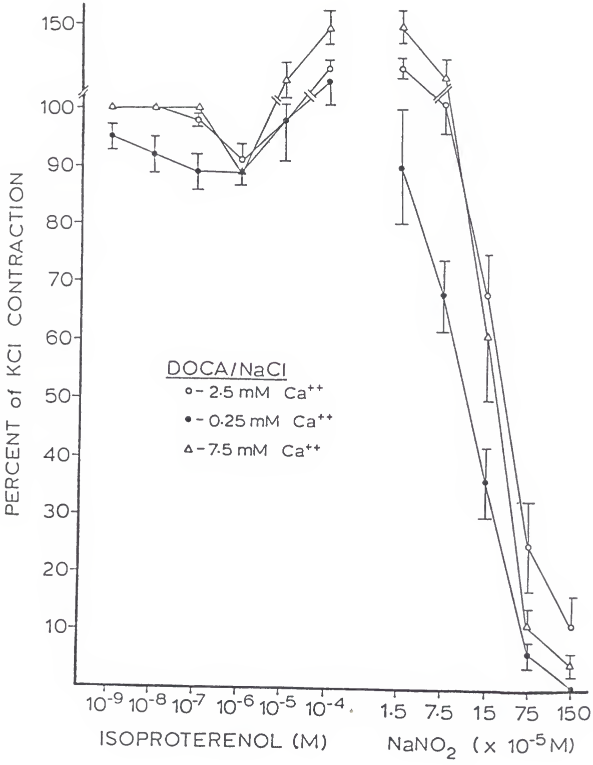

PERCENT of MAXIMAL KCl RESPONSE
100
90
80
70
60
50
40
30
20
10
8
16
24
32
40
60
80
KCl (mM)
DOCA/NaCl
○ – 2·5 mM Ca++
● – 0·25 mM Ca++
△ – 7·5 mM Ca++

Figure 27. Dose-response relationship between concentration of isoproterenol and sodium nitrite and relaxation, expressed as a percent of KCl contraction, of rings of femoral artery from DOCA/NaCl hypertensive rats equilibrated in a low (0.25 mM), normal (2.5 mM), or high (7.5 mm) calcium Krebs physiological solution. No difference in maximal isoproterenol-induced relaxation of vascular smooth muscle was observed between the three levels of calcium. However, an increased sensitivity ($p < .05$) to low concentrations of isoproterenol (10^{-9} to 10^{-7} M) was observed in the low calcium Krebs media. No difference in the maximum response to sodium nitrite was observed. Each point represents the mean and standard error of 12 rings.


150
100
90
80
70
60
50
40
30
20
10
PERCENT of KCl CONTRACTION
DOCA/NaCl
○ - 2.5 mM Ca++
● - 0.25 mM Ca++
△ - 7.5 mM Ca++
10-9 10-8 10-7 10-6 10-5 10-4
ISOPROTERENOL (M)
1.5 7.5 15 75 150
NaNO2 (x 10-5 M)

Experiment II as being the result of α-adrenergic stimulation.

The responsiveness of VSM from controls to ISO was similar to the DOCA/NaCl treated group in terms of the level of extracellular Ca^{++} (Figure 28). That is, in the low Ca^{++} Krebs an increase in the sensitivity to ISO was observed, however, the maximal response was not altered. As opposed to the DOCA/NaCl group, high Ca^{++} did decrease the maximal ISO-induced relaxation in controls. The increase in tension observed at 10^{-4} M ISO was similar in low and normal Ca^{++} but was slightly greater in high Ca^{++}. $NaNO_2$ dose-response curves were similar in both controls and DOCA/NaCl hypertensive rats at all levels of extracellular Ca^{++}. Again, to obtain a better appreciation of the effect of altering extracellular Ca^{++} on VSM responsiveness to NE, the data for each level of Ca^{++} were graphed together. Figure 29 shows the dose-response curves of VSM from controls in the presence of high, normal and low Ca^{++} Krebs solution. It is evident that the low Ca^{++} Krebs results in a slight decrease in both the sensitivity and contractility of rings of femoral artery. In the DOCA/NaCl treated group, however, VSM responsiveness to NE is decreased by approximately 80 percent in the low Ca^{++} Krebs (Figure 30). When the data are expressed as a percent of the maximal NE response, no difference is observed in the response of VSM from controls at any level of Ca^{++} (Figure 31). However, VSM from the DOCA/NaCl hypertensive rat

Figure 28. Dose-response relationship between concentration of isoproterenol and sodium nitrite and relaxation, expressed as a percent of KCl contraction, of rings of femoral artery from control rats equilibrated in a low (0.25 mM), normal (2.5 mM), or high (7.5 mM) calcium Krebs physiological solution. Maximal isoproterenol-induced relaxation of vascular smooth muscle in the high calcium Krebs media was significantly attenuated ($p < .05$) when compared to responses in low and normal calcium Krebs. A significant increase in the sensitivity to low concentrations of isoproterenol (10^{-9} to 10^{-7} M) was observed in the low calcium media. Maximal relaxation in response to sodium nitrite was slightly attenuated ($p < .05$) in the high calcium Krebs. Each point represents the mean and standard error of 12 rings.

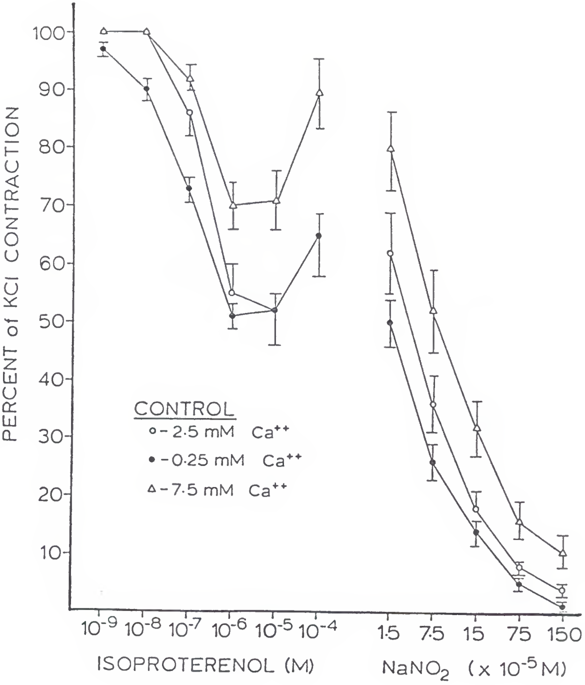

PERCENT of KCl CONTRACTION
100
90
80
70
60
50
40
30
20
10
CONTROL
○ - 2.5 mM Ca++
● - 0.25 mM Ca++
△ - 7.5 mM Ca++
10-9 10-8 10-7 10-6 10-5 10-4
ISOPROTERENOL (M)
1.5 7.5 15 75 150
NaNO2 (x 10-5 M)

Figure 29. Dose-response relationship between concentration of norepinephrine and development of active tension by rings of femoral artery from control rats equilibrated in a low (0.25 mM), normal (2.5 mM), or high (7.5 mM) calcium Krebs physiological solution. A significant decrease ($p < .05$) in the maximal response of vascular smooth muscle to norepinephrine (10^{-6} to 10^{-4} M) was observed in the low calcium Krebs media. Each point represents the mean and standard error of 12 rings.

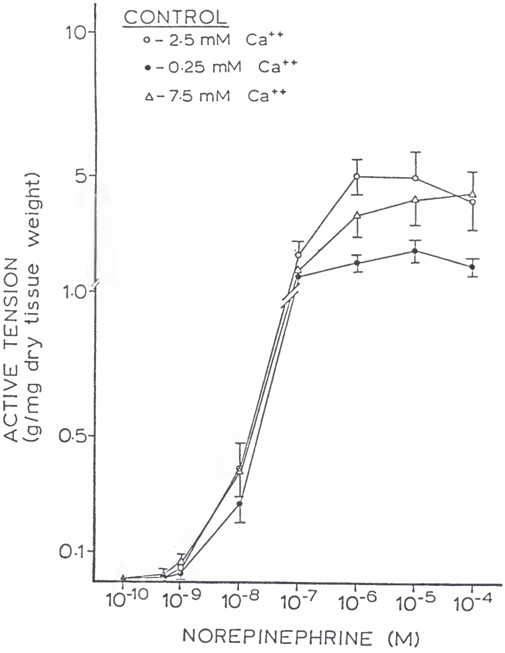

CONTROL
○ - 2·5 mM Ca⁺⁺
● - 0·25 mM Ca⁺⁺
△ - 7·5 mM Ca⁺⁺
ACTIVE TENSION (g/mg dry tissue weight)
10
5
1.0
0.5
0.1
10⁻¹⁰
10⁻⁹
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁵
10⁻⁴
NOREPINEPHRINE (M)

Figure 30. Dose-response relationship between concentration of norepinephrine and development of active tension by rings of femoral artery from DOCA/NaCl hypertensive rats equilibrated in a low (0.25 mM), normal (2.5 mM), or high (7.5 mM) calcium Krebs physiological solution. A significant decrease ($p < .001$) in the response of vascular smooth muscle to all concentrations of norepinephrine was observed in low calcium Krebs media when compared to responses in normal and high calcium Krebs. Each point represents the mean and standard error of 12 rings.

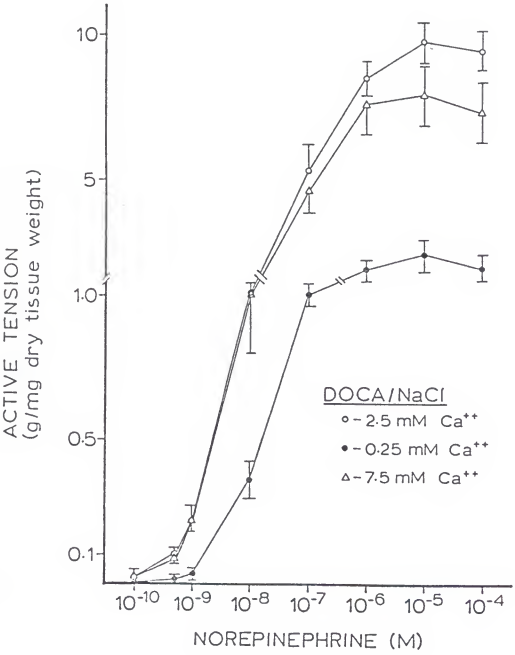
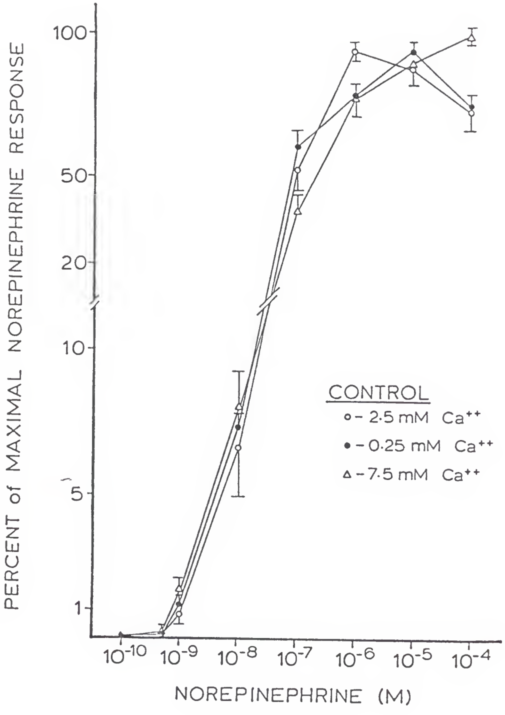

10
5
1.0
0.5
0.1
ACTIVE TENSION
(g/mg dry tissue weight)
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
NOREPINEPHRINE (M)
DOCA/NaCl
○ – 2.5 mM Ca^{++}
● – 0.25 mM Ca^{++}
△ – 7.5 mM Ca^{++}

Figure 31. Dose-response relationship between concentration of norepinephrine and percent of maximal norepinephrine response of rings of femoral artery from control rats equilibrated in a low (0.25 mM), normal (2.5 mM), or high (7.5 mM) calcium Krebs physiological solution. Each response is calculated from the data in Figure 29 and is shown as a percent of the maximum active tension generated. No difference in the response of vascular smooth muscle to norepinephrine was observed between the three levels of calcium. Each point represents the mean and standard error of 12 rings.


PERCENT of MAXIMAL NOREPINEPHRINE RESPONSE
100
50
20
10
5
1
10^-10
10^-9
10^-8
10^-7
10^-6
10^-5
10^-4
NOREPINEPHRINE (M)
CONTROL
○ – 2·5 mM Ca++
● – 0·25 mM Ca++
△ – 7·5 mM Ca++

still exhibit a decrease in the sensitivity to NE stimulation in the low Ca^{++} Krebs (Figure 32). This is further reflected in the increase in the ED10 value for VSM from DOCA/NaCl rats in response to NE. No difference in the ED10 values was evident in controls (Table 2).

Discussion

The results from Experiment II showed that an increase in reactivity to KCl and NE occurred in VSM from DOCA/NaCl hypertensive rats. In addition, the β-adrenergic mediated relaxation induced by ISO was significantly reduced in the DOCA/NaCl treated rat. Several hypotheses have been made as to the possible mechanisms involved in the alterations in vascular reactivity to these agents. One of these, the role of extracellular Ca^{++}, has been investigated in the present experiment.

It is quite evident from the KCl dose-response curves that extracellular Ca^{++} plays a significant role in the alterations of VSM reactivity in DOCA/NaCl hypertensive rats. The fact that the response to KCl was altered more in the DOCA/NaCl treated rat when the level of Ca^{++} was changed suggests that the VSM cell of the DOCA/NaCl hypertensive rat is much more labile to alterations in extracellular Ca^{++} than the control.

The decreased responsiveness of VSM from both groups to KCl in the high Ca^{++} Krebs is most likely due to membrane stabilization (Webb and Bohr, 1978). At high concentrations,

Figure 32. Dose-response relationship between concentration of norepinephrine and percent of maximal norepinephrine response of rings of femoral artery from DOCA/NaCl hypertensive rats equilibrated in a low (0.25 mM), normal (2.5 mM), or high (7.5 mM) calcium Krebs physiological solution. Each response is calculated from the data in Figure 30 and is shown as a percent of the maximum active tension generated. A significant decrease ($p \leq .05$) in the norepinephrine sensitivity (10^{-10} to 10^{-8} M) of vascular smooth muscle was observed in the low calcium Krebs when compared to responses in normal and high calcium Krebs. Each point represents the mean and standard error of 12 rings.

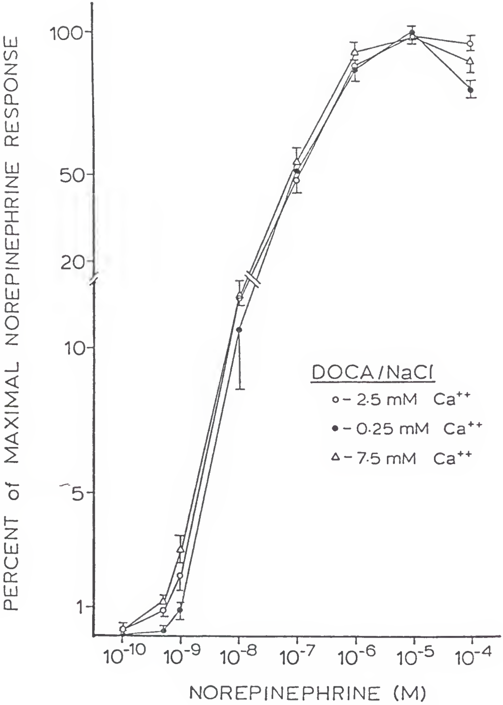

PERCENT of MAXIMAL NOREPINEPHRINE RESPONSE
100
50
20
10
5
1
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
NOREPINEPHRINE (M)
DOCA/NaCl
○ – 2.5 mM Ca^{++}
● – 0.25 mM Ca^{++}
△ – 7.5 mM Ca^{++}

TABLE 2. EFFECTIVE DOSE OF NOREPINEPHRINE WHICH RESULTS IN A 10 PERCENT OF MAXIMAL RESPONSE (ED10), IN RINGS OF FEMORAL ARTERY FROM CONTROL AND DOCA/NaCl HYPERTENSIVE RATS AT VARIOUS CONCENTRATIONS OF EXTRACELLULAR CALCIUM.

Treatment Group	Extracellular Calcium Concentration		
	0.25 mM	2.5 mM	7.5 mM
Control	1.25×10^{-8} $\pm 2.30 \times 10^{-9}$ M[a]	1.3×10^{-8} $\pm 2.1 \times 10^{-9}$ M	1.4×10^{-8} $\pm 2.0 \times 10^{-9}$ M
DOCA/NaCl	8.6×10^{-9} $\pm 2.1 \times 10^{-9}$ M	6.4×10^{-9} $\pm 1.5 \times 10^{-9}$ M[b]	6.2×10^{-9} $\pm 1.4 \times 10^{-9}$ M[b]

[a] Mean ± SEM

[b] Significantly different from control ($p < .05$)

Ca^{++} may have an effect upon the VSM cell to decrease the membrane permeability to monovalent ions, as well as influencing its own ability to permeate the membrane (Hurwitz, 1965). Therefore, the increased extracellular Ca^{++} may actually decrease the response to KCl stimulation because of its effect on the membrane to decrease the influx of extracellular Ca^{++}.

The decrease in response to KCl in the low Ca^{++} Krebs could simply be explained by the fact that the extracellular availability of Ca^{++} is decreased. However, if this were the case, one would expect the responses of both controls and DOCA/NaCl hypertensive rats to be similar. Since it was hypothesized that the increase in reactivity of VSM from DOCA/NaCl rats to KCl was due to an increase in Ca^{++} permeability or sensitivity, then decreasing the concentration of extracellular Ca^{++} should normalize the responses to some degree. However, the response of VSM from the DOCA/NaCl treated rat in low Ca^{++} was significantly reduced when compared to controls and DOCA/NaCl rats in normal Ca^{++}. It is suggested that the alterations in Ca^{++} handling by the VSM cell of the DOCA/NaCl rat are more complex than merely permeability changes.

Studies on VSM from SHR have shown a decrease in ATP-dependent Ca^{++} accumulation (Ca^{++} pump) by plasma membrane fractions (Kwan and Daniel, 1982; Wei _et_ _al_., 1976). Although no studies have been done in the DOCA/NaCl hypertensive rat, it is possible that the increase in responsiveness is due to this membrane defect in Ca^{++} handling. If the VSM cell is unable to maintain normal levels of intracellular Ca^{++} (approximately 10^{-7} M) due to a decrease in the activity of this Ca^{++} pump, then the cell could exist in a state of elevated myogenic tone. In this state, the VSM is more easily activated and exhibits an enhanced response (Fitzpatrick and Szentivanyi, 1980).

Evidence in support of this decrease in Ca^{++} pump activity comes from the KCl washout curves. The $t_{\frac{1}{2}}$ values were significantly higher in VSM from the DOCA/NaCl group at all levels of extracellular Ca^{++} when compared to controls. This suggests a decrease in the ability of the VSM to sequester Ca^{++} intracellularly or pump Ca^{++} to the extracellular space. Field et al. (1972) have observed similar results in aortic rings of SHR. It has been suggested that the slower rate of relaxation following washout of KCl in SHR aortic smooth muscle is due to a decrease in membrane Ca^{++} pump activity (Wei et al., 1976).

Recent evidence has shown the existence of low and high affinity Ca^{++} binding sites in VSM. These sites appear to be involved in the mobilization of extracellular Ca^{++} upon stimulation of the VSM (Hester and Weiss, 1981). Based upon ^{45}Ca uptake studies, Weiss (1982) has suggested that responses of VSM to KCl depolarization involve the release of Ca^{++} from the low affinity binding sites. Also, in a low calcium media, the binding of Ca^{++} to high affinity sites is favored over low affinity sites, and thus the response to KCl is diminished (Goodman et al., 1972). From these results it may be postulated that the number of low affinity Ca^{++} binding sites is increased in VSM of DOCA/NaCl hypertensive rats. This would account for the increased response of VSM from DOCA/NaCl rats at normal levels of calcium, while the response to KCl in low calcium is greatly reduced.

Several mechanisms were discussed in Experiment II concerning alterations in VSM β-adrenergic responsiveness in DOCA/NaCl hypertensive rats. It was postulated that one of the mechanisms that may be involved in the decrease in ISO-induced relaxation is an increase in Ca^{++} permeability or an alteration in the handling of Ca^{++} by the VSM cell. From the present data it appears that this is not the case. Although an increase in the sensitivity to ISO occurred in the low Ca^{++} Krebs, it was very much apparent that the maximal response was not altered. The results in VSM from controls were quite similar to those observed in the DOCA/NaCl treated rat. That is, the sensitivity to ISO was increased in low Ca^{++} but the maximal response was not. Therefore, it is suggested that the decrease in β-adrenergic responsiveness is most likely due to an alteration at the level of the β-receptor (decrease in number and/or affinity), or in the activation of the cyclic AMP system and not necessarily due to an alteration of intracellular Ca^{++} sequestration or plasma membrane Ca^{++} pump activity. The findings of Woodcock et al. (1980)

support this hypothesis in that they showed a decrease in the number of β-adrenergic receptors in VSM of DOCA/NaCl hypertensive rats. This is not to say conclusively that an alteration in Ca^{++} handling by the VSM cell of DOCA/NaCl hypertensive rats is not involved in the decrease in β-adrenergic responsiveness. However, it appears that any alterations that may exist are not involved to a great extent in this decreased response. Also, since VSM from DOCA/NaCl treated rats responded to $NaN0_2$ in a fashion similar to controls, it can be suggested that the relaxation process, per se, is not appreciably affected.

Many studies have been done on the SHR dealing with the role of extracellular Ca^{++} in the increased reactivity of VSM to NE (Fitzpatrick and Szentivanyi, 1980; Aoki et al., 1980; Mulvany and Nyborg, 1980, for example). However, few studies have been done in the DOCA/NaCl hypertensive rat (Hinke, 1966; Pang and Sutter, 1980).

In the present study, it is quite evident that extracellular Ca^{++} plays an important role in VSM responsiveness to NE just as it did for KCl. Based upon these findings it is apparent that the increase in NE sensitivity of VSM from DOCA/NaCl hypertensive rats is largely due to an increase in the Ca^{++} sensitivity of the VSM cell. By decreasing the level of extracellular Ca^{++} the increased reactivity could be abolished and the response normalized to that of the control.

The specific mechanism(s) behind the role for the increased Ca^{++} sensitivity resulting in an increase in NE sensitivity is yet to be determined. One possible mechanism may involve an alteration in membrane Ca^{++} binding sites similar to that proposed for the alteration in KCl responsiveness. Weiss (1982) has proposed that release of Ca^{++} from high affinity sites occurs upon stimulation of VSM with NE and that this Ca^{++} enters the cell to contribute to the NE-induced contraction. Hester and Weiss (1981) also suggest that NE may stimulate the release of Ca^{++} from low affinity sites as well. The contribution of each of these to the total amount of Ca^{++} released upon NE stimulation is unknown. As was mentioned earlier, binding of Ca^{++} to the high affinity sites is favored in low Ca^{++} solutions. If NE stimulates the influx of extracellular Ca^{++} by releasing Ca^{++} from both high and low affinity sites then the decrease in NE responsiveness in low Ca^{++} could possibly be explained. In conjunction with the hypothesized increase in low affinity binding sites in VSM from DOCA/NaCl hypertensive rats, a decrease in high affinity sites may also occur. Since low Ca^{++} solution favor binding to high affinity sites, the amount of Ca^{++} that would be available for release upon stimulation would be decreased. This would result in a decrease in the response to NE.

Another possible mechanism for the increase in NE sensitivity comes from recent studies that show VSM contraction in response to NE is due to stimulation of postsynaptic α_1 and α_2-adrenergic receptors. Bentley et al. (1977) were the first to observe that a subpopulation of α-adrenergic receptors mediated a vasoconstriction not blocked by the selective α_1-adrenergic antagonist prazosin. It was not until 1979, however, that Timmermans et al. were able to pharmacologically classify the receptor as α_2-adrenergic. Further studies (van Zwieten et al., 1982; Cauvin et al., 1982; van Meel et al., 1983) have shown that vasoconstriction due to α_2-adrenergic receptor stimulation requires the influx of extracellular Ca^{++}. It was also shown that the role for extracellular Ca^{++} in NE responses increases when progressing from larger conduit arteries to resistance vessels (Cauvin et al., 1982).

Since activation of α_2-adrenergic receptors involves the influx of extracellular Ca^{++}, the increase in NE sensitivity of VSM from DOCA/NaCl hypertensive rats may be the result of an increase in the number and/or affinity of α_2-adrenergic receptors. On the other hand, no alterations in the receptor itself may occur, however, if the VSM cell is more permeable to Ca^{++}, then subsequent activation of the α_2-adrenergic receptor would result in an increase in NE responsiveness. Lowering the amount of Ca^{++}, unfortunately, does not distinguish between the two possibilities. In addition, since membrane Ca^{++} binding sites are involved in NE stimulation of VSM with resultant influx of extracellular Ca^{++}, it is also possible that an alteration in these binding sites may affect the response of α_2-adrenergic stimulation.

In summary, the increased responsiveness of VSM from DOCA/NaCl hypertensive rats to KCl and NE appears to be largely due to an increased Ca^{++} sensitivity. The exact mechanism(s) behind the increase remains to be elucidated. The decrease in β-adrenergic responsiveness, however, does not appear to involve a defect in the handling of Ca^{++} (sequestration of extrusion) by the VSM cell since the ability of the VSM to respond to a nonspecific smooth muscle relaxant ($NaN0_2$) is not altered. A defect in the β-adrenergic system upstream from the actual sequestration of Ca^{++} seems to be the cause of the decreased response.

PART IV

VASCULAR SMOOTH MUSCLE Na,K-ATPase (SODIUM-POTASSIUM PUMP) IN DOCA/NaCl HYPERTENSIVE RATS

Introduction

The membrane bound enzyme adenosine triposphatase was first demonstrated in squid axon and rat peripheral nerves by Libet (1948) and Abood and Gerard (1954). Further studies showed that (a) energy for active transport of sodium across cell membranes comes from adenosine triphosphate (Caldwell and Keynes, 1957), (b) there is coupling between active outward transport of sodium and inward transport of potassium (Glynn, 1956), and (c) active transport of sodium is inhibited by cardiac glycosides (Glynn, 1957). The presence of sodium, potassium-activated adenosine triphosphatase (Na,K-ATPase; sodium pump) has since been demonstrated in a number of tissues. It was not until 1972, however, when Wolowyk *et al*. were able to clearly show that a ouabain-sensitive Na,K-ATPase existed in VSM.

In 1957, Leonard observed that equilibration of helically cut strips of rabbit carotid artery in potassium-free Krebs solution or in Krebs solution containing a cardiac glycoside resulted in a slight contraction of the tissue. Leonard suggested that there was an alteration in the intracellular electrolytes, particularly potassium, that lead to the increase in tension. A number of investigators (Dawes, 1941; Emanuel *et al*., 1959; Konold *et al*., 1968) have observed similar effects of potassium and ouabain on VSM. In 1972, Chen *et al*. proposed that the mechanism by which potassium and ouabain altered the tone of VSM was through a change in the activity of Na,K-ATPase. Based on prior *in vitro* experimentation, they hypothesized that if potassium was removed from the bathing medium of an *in vitro* preparation of VSM the activity of the sodium-potassium pump would be decreased. This would result in an accumulation of positive charges (sodium ion) inside the cell leading to depolarization and contraction of the tissue. On the other hand, if extracellular potassium was increased slightly above normal levels (4 mEq/l in the rat) relaxation of the tissue would occur due to hyperpolarization caused by a decrease in the net positive charges in the cell. That is, the activity of the sodium-potassium pump was enhanced resulting in decreased levels of intracellular sodium. They also hypothesized that since ouabain inhibits the sodium-potassium pump, the effects would be similar to a potassium-free medium which causes an increase in intracellular sodium and depolarization of the tissue. Using an *in vivo* preparation of canine gracilis artery, Chen and his colleagues showed that an infusion of a potassium-free solution resulted in an increase in resistance to blood flow. An increase in resistance was also observed with an infusion of ouabain while a high potassium solution decreased resistance. Ouabain also blocked the decrease in resistance seen with the high potassium solution.

Although investigators agree that the sodium-potassium pump is involved in changes seen in vascular tone there is much controversy over the exact mechanism(s) through which it mediates these changes. Three mechanisms have been implicated. The first involves the electrogenic aspect of the sodium-potassium pump and changes in membrane potential. As discussed earlier, Chen *et al.* (1972) observed changes in resistance to blood flow by increasing or decreasing the potassium concentration of the perfusion medium. According to their hypothesis, low concentrations of potassium caused a depolarization and contraction of the artery while high concentrations of potassium resulted in hyperpolarization and relaxation. Inhibition of the sodium-potassium pump by ouabain also resulted in depolarization and contraction of VSM. With the use of microelectrodes, Hendrickx and Casteels (1974) were able to measure changes in the membrane potential of rabbit ear artery cells when exposed to various solutions. They were able to show that a potassium-free solution caused a decrease in membrane potential (depolarization) followed by an increase in the tension of the artery. When potassium was added back to the medium, this resulted in a hyperpolarization and relaxation of the artery which could be blocked by ouabain. Ouabain (10^{-5} M) also induced a contraction in the VSM which was preceded by a decrease in membrane potential. They proprosed that the increase in tension is due to an increase in intracellular calcium by release from intracellular stores or by entry from the extracellular compartment as a direct result of the depolarization of the cell.

The second mechanism by which the sodium-potassium pump may regulate VSM tone involves a sodium-calcium exchange across the cell membrane. Bohr et al. (1969) observed that ouabain (10^{-5} M) caused an increase in tone of both mesenteric artery and aortic strips. It was suggested that inhibition of the sodium-potassium pump by ouabain resulted in an increase of intracellular sodium which stimulated a sodium-calcium exchange mechanism similar to that described for squid axon. This exchange would elevate the concentration of ionized calcium in the cell resulting in an increase in tension. In the absence of external calcium no change in the vascular tone of rat tail artery (Friedman et al., 1973) or guinea pig aorta (Ozaki et al., 1978) in response to ouabain was observed. Toda (1980) found that the contractile response of various arteries to ouabain varied inversely with the period of exposure to a calcium-free medium. These results suggest that the ouabain and potassium-free induced contractions are associated with the influx of calcium across the cell membrane independent of changes in membrane potential. This is supported by the fact that the calcium antagonists verapamil and nifedipine blocked the ouabain induced contractions in certain vessels (Toda, 1980). In opposition to Hendrickx and Casteels, Reiner (1978) observed little change in the membrane potential of rabbit ear artery when exposed to a low potassium medium. Lang and Blaustein (1980) support this finding and therefore suggest that the increase in intracellular calcium is due mainly to the sodium-calcium exchange mechanism and not membrane depolarization. Ozaki et al. (1978) also feel that the increase in tone cannot be solely explained by depolarization of the VSM since the onset of contraction is slow while depolarization of the membrane due to inhibition of the sodium-potassium pump is almost instantaneous.

The third mechanism by which the sodium-potassium pump may regulate VSM tone is through the release of endogenous catecholamines. Bogdanski and Brodie (1969) observed a decrease in norepinephrine content of the rat heart when exposed to a potassium-free solution or cardiac glycoside. From this Karaki and Urakawa (1977) and Bonaccorsi et al. (1979) have suggested that the increase in tone observed in the presence of low concentrations of potassium or a cardiac glycoside may be the result of the release of endogenous catecholamines from sympathetic nerve endings. Using rabbit thoracic aortic strips Karaki and Urakawa were able to block ouabain-induced contractions with α-adrenergic blockers, mechanical denervation, or reserpine pretreatment. Bonaccorsi obtained similar results with the rat tail and femoral arteries, and dog mesenteric artery. Karaki et al. (1978) observed phentolamine inhibition of ouabain and potassium-free induced contractions but also noticed species differences in the sensitivity to ouabain. Using a radioenzymatic method, Palaty (1981) was able to show a gradual increase in the rate of norepinephrine released from the rat tail artery preparation in the presence of potassium-free and ouabain containing solutions, and was able to block the release with cocaine and phenoxybenzamine.

In recent years, several methods have been developed and used to measure sodium-potassium pump activity. Based upon the observations of Chen et al. (1972), one of these methods, potassium-induced relaxation of VSM, was developed by Webb and Bohr (1978) as a functional index of the vascular sodium-potassium pump. Since then, several studies using this method have been done measuring sodium-potassium pump activity in VSM of various animals (Webb et al., 1983a; Bukoski et al., 1983).

Another measure of the vascular sodium-potassium pump involves the use of ^{3}H-ouabain binding (Deth and Lynch, 1980). Although this method does not measure pump activity, it gives an indication of the number of pump sites.

A third method was adapted from the rubidium-86 (^{86}Rb) uptake technique used for estimating pump activity in various tissues (Bernstein and Israel, 1970; Ku et al., 1974). Using the radioisotope rubidium-86, Overbeck et al. (1976) initially used this technique on VSM to assess sodium-potassium pump activity in one-kidney, one-wrapped hypertensive dogs. Ouabain-sensitive as well as ouabain-insensitive uptake was measured. Ouabain-sensitive uptake reflects active transport while ouabain-insensitive uptake is a measure of passive ion movement.

The causes for the increase in vascular resistance seen in hypertension are not well defined due to the number of changes that occur in the vasculature. Recent studies in humans and various forms of experimental hypertension have shown that one of these changes is an alteration in the activity of the sodium-potassium pump (Haddy et al., 1978).

Garay et al. (1980) reported an increase in sodium-potassium pump activity of erythrocytes from essential hypertensive patients and suggested that this alteration is associated with an inherited defect of the sodium-potassium co-transport.

In renal hypertensive dogs, Overbeck (1972) observed a decrease in the vasodilator response to infused potassium. Although the presence of the sodium-potassium pump in VSM had not been clearly demonstrated at that time, he suggested a basic defect in the operation of the pump. Using isolated hindlimb preparations of genetic and renal hypertensive rats, Overbeck and Clark (1975) saw an attenutation of the vasodilator response to potassium in the renal hypertensive rat, however, the genetic hypertensive rat showed no difference in response when compared to the normotensive rat. Sodium-potassium pump activity in VSM of dogs and rats with various forms of experimental hypertension was assessed by using ouabain-sensitive ^{86}Rb uptake (Haddy *et al*., 1978; Pamnani *et al*., 1980a; Pamnani *et al*., 1980b). Suppression of sodium-potassium pump activity was observed in the nongenetic low renin forms of hypertension (DOCA/salt; one-kidney, one clip) while the genetic hypertensive animals (SHR; Dahl salt-sensitive) showed an increase in pump activity. Overbeck *et al*. (1981) also showed an increase in pump activity in the Dahl salt-sensitive rat. However, there was also an increase in passive permeability of the VSM cell to rubidium which may suggest an increase in the permeability to sodium. Friedman and Friedman (1976) have also observed an increase in the passive permeability of the SHR tail artery to certain cations. With the increase in permeability to sodium, intracellular levels of sodium may rise to the point where an increase in the activity of the pump occurs, as observed in the SHR. Whereas Pamnani *et al*. (1980a, 1980b) have observed pump suppression in the DOCA/salt hypertensive rat, Friedman *et al*. (1975) have shown an increase in sodium-potassium pump activity. Friedman suggests that this is due to an increase in permeability of the cell to sodium and that DOCA, like aldosterone, increases the synthesis of Na,K-ATPase.

Furthermore, Brock et al. (1982) also observed an increase in ^{86}Rb uptake in rat aorta and tail artery of DOCA/salt and Grollman renal hypertensive animals. A sigmoid relationship between total cell sodium and ouabain-sensitive ^{86}Rb uptake was seen in both control and hypertensive animals, however, the activity of the sodium-potassium pump for a given level of cell sodium in the hypertensive animals was greater than controls. This suggests that in the hypertensive animal, differences in pump activity may not simply reflect differences in membrane permeability and that other factors may be involved such as increases in the number of active pump sites. Utilizing two forms of Goldblatt hypertension in the rat (one-kidney, one clip and two-kidney, one clip) Overbeck and Grissette (1982) showed an increase in vascular sodium-potassium pump activity in both the tail artery and aorta of hypertensive animals when compared to controls. In opposition to Brock et al. (1982), however, the increases in activity were not related to an altered renin-angiotensin-aldosterone system, changes in body fluid volumes or intracellular sodium concentration.

Using the potassium-relaxation method, Webb (1982) showed that femoral arteries from DOCA-hypertensive pigs exhibited an increase in pump activity when compared to controls. Potassium relaxation of VSM from two-kidney, one clip renal hypertensive rats (Webb et al., 1983a) and one-kidney, one clip hypertensive rabbits (Hagen et al., 1982) was also significantly greater than controls.

To assess whether or not elevated intravascular pressure had an effect on sodium-potassium pump activity, Overbeck et al. (1982) measured ^{86}Rb uptake in the hypertensive and normotensive portions of aorta from rats with chronic coarctation hypertension. They observed similar increases in ^{86}Rb uptake for both the normotensive and hypertensive portions of the aorta and suggest that alterations in pump activity are not the direct result of increased pressure. Alterations in pump activity also could not be attributed to increased concentrations of intracellular sodium or changes in body fluid volume.

Recently, investigators have proposed that a circulating inhibitor of the sodium-potassium pump may be involved in the pathogenesis of certain forms of experimental and genetic hypertension (de Wardener and MacGregor, 1980; MacGregor and de Wardener, 1981). Dahl et al. in 1969 was the first to suggest the presence of a saluretic substance which caused an increase in blood pressure. Pamnani et al. (1981a, 1981b) isolated a heat-stable ouabain-like humoral factor from the plasma of hypertensive rats (low renin form) which decreased vascular Na,K-ATPase activity as determined by ^{86}Rb uptake. Pamnani et al. (1982) also have shown that the transmembrane potential of vascular smooth muscle cells in caudal arteries from one-kidney, one clip hypertensive rats is significantly lower than controls. Boiled plasma supernatants from these hypertensive rats depolarized muscle cells in normotensive caudal arteries while boiled plasma supernatants from normotensive rats had no effect on the membrane potential. The effect of ouabain on smooth muscle cells was similar to the boiled plasma supernatants from hypertensive animals, suggesting the presence of a ouabain-like humoral substance in this form of hypertension.

Kojima _et_ _al_. (1982) observed a marked decrease in blood pressure in DOCA/salt hypertensive rats, which lasted for approximately one hour, following the administration of anti-digoxin antibody. The antibody had no effect on normotensive animals.

Recent evidence has suggested that the synthesis or secretion of the ouabain-like factor may be under the control of the periventricular tissue of the anteroventral portion of the third ventricle (AV3V) of the brain. Lesions of the AV3V region prevent the development of DOCA/salt hypertension and also prevent the suppression of sodium-potassium pump activity in tail arteries incubated in plasma from lesioned rats treated with DOCA/salt (Songu-Mize et al., 1982).

The AV3V region also appears to be involved in the development of two-kidney Goldblatt hypertension (Songu-Mize _et_ _al_., 1983), however, Gordon _et_ _al_. (1982) showed that the development of hypertension in SHR does not depend upon the integrity of this region.

From the preceding discussion it is evident that the sodium-potassium pump plays a role in VSM tone and is involved in the pathogenesis of various forms of experimental hypertension. However, there are conflicting reports regarding alterations in sodium-potassium pump activity. Therefore, in the second part of this dissertation, experiments will be reported which determined sodium-potassium pump activity in VSM from control and DOCA/NaCl hypertensive rats.

Three methods to determine alterations in vascular pump activity were used. Two of these, potassium relaxation and ^{86}Rb uptake have been mentioned briefly in the introduction to this section. The third involves the "membrane-stabilizing" effect of calcium. Calcium is known to cause a relaxation of VSM at high concentrations, which appears to be mediated by the sodium-potassium pump since ouabain prevented the relaxant effect of calcium (Webb and Bohr, 1978). Further studies (Webb and Bohr, 1980) showed that higher levels of calcium were required to induce a relaxation of tail arteries from SHR when compared to controls, while relaxation in response to manganese was not affected. This provided evidence that the "membrane stabilizing" effect of calcium could be used as an index of abnormal VSM function in hypertensive animals.

Therefore, in addition to the potassium-relaxation experiment and the ^{86}Rb uptake assay, a series of experiments utilizing the "membrane stabilizing" effect of calcium were done. Since this effect is dependent upon the sodium-potassium pump activity, the response to calcium was carried out at differing levels of pump activity. That is, the response was observed in the presence of ouabain and at various levels of extracellular potassium ion concentration. Since sodium-potassium pump activity is dependent upon the extracellular potassium ion concentration, and the relaxation induced by calcium is mediated by the sodium-potassium pump, then the response to calcium should be potassium concentration-dependent. Any differences in vascular pump activity between controls and DOCA/NaCl hypertensive rats should be evident from these three studies.

Experiment IV

This experiment was designed to investigate alterations in VSM Na,K-ATPase (sodium-potassium pump) activity in DOCA/NaCl hypertensive rats using potassium relaxation of rings of femoral artery as the index.

Methods

Twelve 6 week-old male S-D rats were used in this experiment. DOCA/NaCl hypertension was induced in 6 of these rats as described in the General Methods section. At 10 weeks of age (4 weeks postimplant) all animals were sacrificed and rings of femoral artery prepared. The tissues were equilibrated for 90 minutes in a low (0.6 mM) potassium Krebs solution to depress the sodium-potassium pump. The Krebs solution was changed every 15 minutes during the equilibration period. At the end of the equilibration period, 20 ml of fresh Krebs solution was placed in the baths and the tissues maximally stimulated with NE (10^{-4} M). After the contraction had stabilized, KCl was added back to the bath in increments of 0.8 mM. This caused a stimulation of the sodium-potassium pump, hyperpolarized the femoral smooth muscle (Hendrickx and Casteels, 1974) and resulted in a relaxation, expressed as a percent of the NE contraction. Each dose of KCl remained in contact with the tissue for a 3 minute period of time.

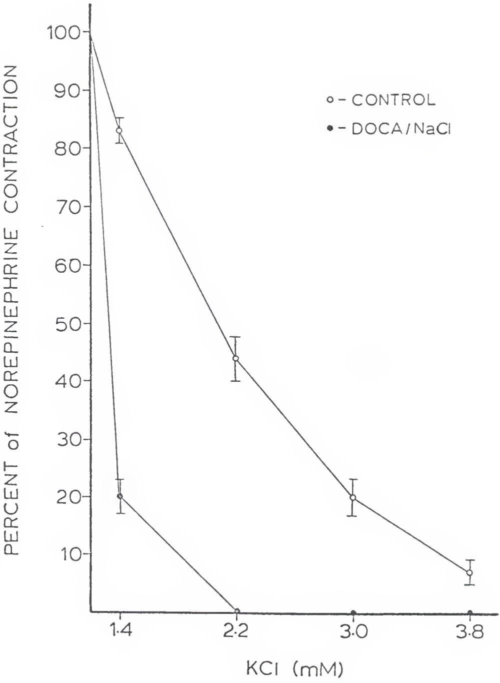
Results

At 1.4 mM KCl (0.6 mM KCl in the Krebs plus 0.8 mM added) rings of femoral artery from DOCA/NaCl hypertensive rats relaxed 80 percent, while those from controls relaxed only 17 percent (Figure 33). Maximum relaxation (100 percent) in femoral smooth muscle from DOCA/NaCl treated rats occurred at 2.2 mM KCl. At this point controls had relaxed only 55 percent. Femoral smooth muscle from controls relaxed 80 percent in response to 3.0 mM KCl and 93 percent in 3.8 mM KCl.

Experiment V

This experiment was designed to investigate alterations in VSM Na,K-ATPase (sodium-potassium pump) activity in DOCA/NaCl hypertensive rats using the rubidium-86 uptake technique as the index.

Figure 33. Dose-response relationship between concentration of KCl and relaxation, expressed as a percent of norepinephrine contraction, of rings of femoral artery from control and DOCA/NaCl hypertensive rats equilibrated in a low potassium (0.6 mM) Krebs physiological solution. Potassium-induced relaxation of vascular smooth muscle from DOCA/NaCl hypertensive rats was significantly greater ($p < .001$) than controls at all concentrations of potassium. Each point represents the mean and standard error of 12 rings.


PERCENT of NOREPINEPHRINE CONTRACTION
100
90
80
70
60
50
40
30
20
10
○ - CONTROL
● - DOCA/NaCl
1·4
2·2
3·0
3·8
KCl (mM)

Methods

Thirty-six 6 week-old male S-D rats were used in this experiment. DOCA/NaCl hypertension was induced in 18 of these rats as described in the General Methods. At 10 weeks of age (4 weeks postimplant) all animals were sacrificed and both femoral arteries quickly removed and placed in an aerated modified Krebs solution at room temperature. The arteries were incubated at 4° C in a potassium-free Krebs solution for 10 minutes to depress sodium-potassium pump activity and load the cells with sodium. One artery was then incubated in a potassium-free Krebs solution with "cold" rubidium chloride (RbCl; 2 mM), ^{86}RbCl (0.1 mM, New England Nuclear; specific activity 2.31 mCi/mg) and ouabain (10^{-4} M) at 37° C. The other artery was incubated in a similar medium but without ouabain. Tissues were incubated for either 3, 10, or 20 minutes to obtain a time course of uptake. Following the incubation period the tissues were washed for 30 seconds with a potassium-free Krebs solution containing 2 mM RbCl. The tissues were allowed to dry to a constant weight (Cahn electrobalance), placed in disposable test tubes and counted using a model 1195 Searle gamma counter.

Results

A significant increase in ouabain-sensitive rubidium-86 intake was observed in femoral arteries from DOCA/NaCl hypertensive rats at all 3 incubation time periods (Table 3). No difference in ouabain-insensitive uptake was seen. Expressing the uptake as pmoles/mg tissue weight/minute at each time interval shows that femoral artery from the DOCA/NaCl hypertensive rat has a faster rate of uptake initially and then decreases toward control values. No difference in ouabain-insensitive uptake was observed.

Experiment VI

This experiment was designed to investigate alterations in VSM Na,K-ATPase (sodium-potassium pump) activity in DOCA/NaCl hypertensive rats using calcium relaxation of rings of femoral artery as the index.

Methods

Sixty 6 week-old male S-D rats were used in this experiment. DOCA/NaCl hypertension was induced in 30 of these rats as described in the General Methods section. At 10 weeks of age (4 weeks postimplant) all animals were sacrificed and rings of femoral artery prepared. The tissues were equilibrated for 90 minutes in a low calcium (0.25 mM) Krebs physiological solution with either 0 mM, 3.0 mM (low), 5.9 mM (normal), or 10 mM (high) potassium. The Krebs solution was changed every 15 minutes during the equilibration period. At the end of the equilibration period, 20 ml of fresh Krebs solution was placed in the baths and the tissues maximally stimulated with NE (10^{-4} M). After the contraction had

TABLE 3. OUABAIN-SENSITIVE (O-S) AND OUABAIN-INSENSITIVE (O-I) RUBIDIUM-86 UPTAKE (pmoles/mg DRY TISSUE WEIGHT) IN FEMORAL SMOOTH MUSCLE FROM CONTROL AND DOCA/NaCl HYPERTENSIVE RATS.

Treatment Group		Incubation Time in 0.1 mM ^{86}Rb		
		3 min.	10 min.	20 min.
Control	O-S	228.31 ± 6.36[a] (76.13 ± 2.12)[b]	768.19 ± 32.56 (76.82 ± 3.26)	1247.64 ± 33.27 (62.38 ± 1.66)
	O-I	95.70 ± 3.34 (31.90 ± 1.12)	146.75 ± 7.73 (14.68 ± 0.77)	173.90 ± 5.69 (8.70 ± 0.29)
DOCA/NaCl	O-S	332.61 ± 17.67[c] (110.87 ± 5.85)[c]	952.98 ± 37.97[c] (95.30 ± 3.80)[c]	1525.27 ± 98.81[c] (76.26 ± 4.94)[c]
	O-I	88.22 ± 2.53 (29.41 ± 0.85)	132.33 ± 7.57 (13.23 ± 0.75)	178.17 ± 7.98 (8.91 ± 0.40)

[a] Mean ± SEM

[b] Values in parenthesis represent uptake expressed as pmoles/mg dry tissue weight/minute

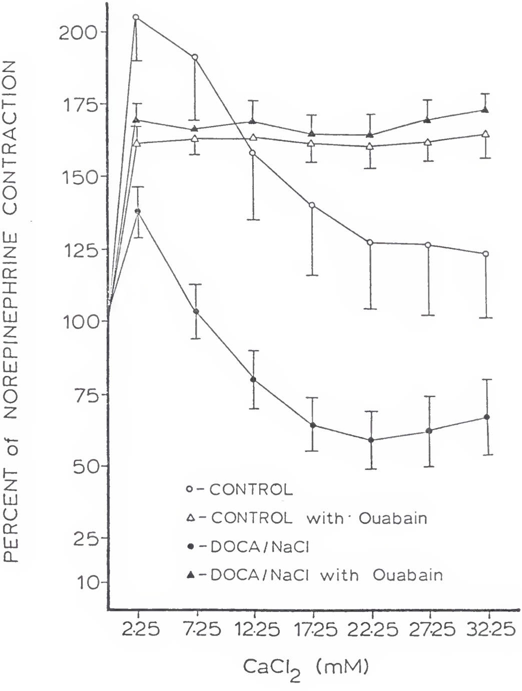
[c] Significantly different from controls ($p < .01$)

stabilized, a dose-response curve (2.25 to 32.25 mM) to calcium chloride ($CaCl_2$) was generated. In one series of experiments tissues were equilibrated in a low calcium, normal potassium Krebs, and one minute prior to NE contraction, ouabain (10^{-4}) was added to the baths. The dose-response curve to $CaCl_2$ was then done following the addition of NE. Responses were expressed as a percent of the NE contraction and as a percent of the NE contraction in 2.25 mM calcium. The tissues remained in contact with each concentration of $CaCl_2$ for a 3 minute period of time. Adjustments in $NaHCO_3$ concentration were not made since precipitation of calcium carbonate occurred only at the high concentrations of $CaCl_2$ and this did not appear to affect the maximal response (based upon preliminary studies.)

Results

The response of femoral smooth muscle to increasing concentrations of $CaCl_2$ in a low calcium (0.25 mM), normal potassium (5.9 mM) Krebs physiological solution in the presence or absence of ouabain is shown in Figure 34. An increase in tension was observed in all groups at 2.25 mM $CaCl_2$. Controls responded maximally with a 110 percent increase in tension above the NE response, followed by DOCA/NaCl in the presence of ouabain (70 percent), control in the presence of ouabain (65 percent) and DOCA/NaCl (35 percent). Femoral smooth muscle from control and DOCA/NaCl hypertensive rats responded to $CaCl_2$ concentrations

Figure 34. Dose-response relationship between concentration of $CaCl_2$ and response, expressed as a percent of norepinephrine contraction, of rings of femoral artery from controls and DOCA/NaCl hypertensive rats equilibrated in a low calcium (0.25 mM) Krebs physiological solution in the presence or absence of ouabain (10^{-4} M). A significant difference ($p < .05$ to $p < .001$) in the response of vascular smooth muscle to increasing concentrations of $CaCl_2$ was observed between the two groups. In the presence of ouabain, no differences were seen. Each point represents the mean and standard error of 12 rings.


PERCENT of NOREPINEPHRINE CONTRACTION
200
175
150
125
100
75
50
25
10
2·25
7·25
12·25
17·25
22·25
27·25
32·25
CaCl$_2$ (mM)
○ - CONTROL
△ - CONTROL with Ouabain
● - DOCA/NaCl
▲ - DOCA/NaCl with Ouabain

of 7.25 mM and greater with a decrease in tension. Ouabain prevented this relaxation in both groups. Expressing the data as a percent of the NE response in 2.25 mM $CaCl_2$ (Figure 35) reveals that the response of femoral smooth muscle from DOCA/NaCl hypertensive rats is significantly greater at $CaCl_2$ concentrations of 7.25 to 27.25 mM.

Rings of femoral artery from control and DOCA/NaCl hypertensive rats equilibrated in a low calcium (0.25 mM) Krebs solution with either 0 mM, 3.0 mM, or 10 mM potassium showed an increase in tension, above the NE response, at 2.25 mM $CaCl_2$ (Figures 36, 37, and 38, respectively). A further increase in tension was observed at 7.25 and 12.25 mM $CaCl_2$ in the 0 mM potassium Krebs, with the maximal response being greater in the DOCA/NaCl rat (730 percent vs. 580 percent). In 3.0 mM and 10 mM potassium Krebs, controls exhibited a greater increase in tension, above the NE response (85 and 200 percent,) respectively) to 2.25 mM $CaCl_2$ than DOCA/NaCl rats (18 and 75 percent, respectively). At concentrations greater than 2.25 mM $CaCl_2$, a decrease in tension was observed in both groups in 3.0 mM and 10 mM potassium Krebs. A small decrease in tension occurred in femoral smooth muscle from control and DOCA/NaCl hypertensive rats in 0 mM potassium Krebs at concentrations greater than

Figure 35. Dose-response relationship between concentration of $CaCl_2$ and relaxation, expressed as a percent of norepinephrine contraction in 2.25 mM calcium, of rings of femoral artery from control and DOCA/NaCl hypertensive rats equilibrated in low calcium (0.25 mM) Krebs physiological solution in the presence or absence of ouabain (10^{-4} M). Each response is calculated from the data in Figure 34 and is shown as a percent of the response at 2.25 mM $CaCl_2$. Calcium-induced relaxation of vascular smooth muscle from DOCA/NaCl hypertensive rats was significantly greater ($p < .05$) than controls. Ouabain prevented the relaxation in both groups. Each point represents the mean and standard error of 12 rings.

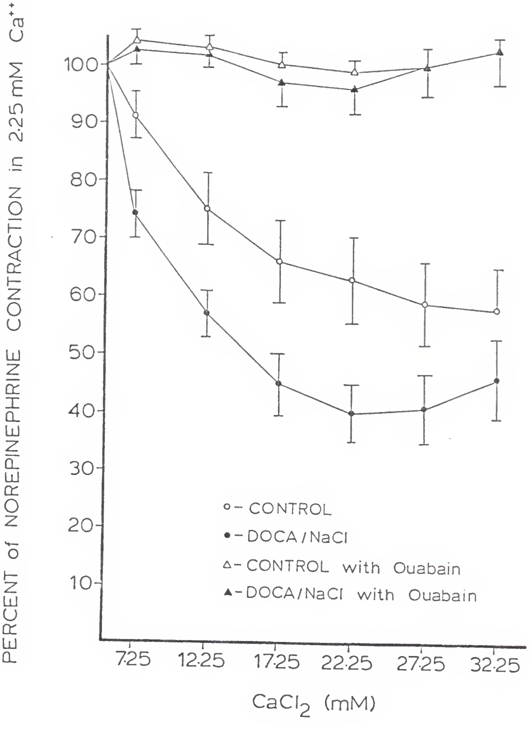

PERCENT of NOREPINEPHRINE CONTRACTION in 2·25 mM Ca++
100
90
80
70
60
50
40
30
20
10
7·25
12·25
17·25
22·25
27·25
32·25
CaCl2 (mM)
○ - CONTROL
● - DOCA / NaCl
△ - CONTROL with Ouabain
▲ - DOCA / NaCl with Ouabain

Figure 36. Dose-response relationship between concentration of $CaCl_2$ and response, expressed as a percent of norepinephrine contraction, of rings of femoral artery from control and DOCA/NaCl hypertensive rats equilibrated in a low calcium (0.25 mM), potassium-free Krebs physiological solution. The response of vascular smooth muscle from DOCA/NaCl hypertensive rats was significantly greater ($p < .05$) than controls at all concentrations of $CaCl_2$ except 32.25 mM. Each point represents the mean and standard error of 12 rings.

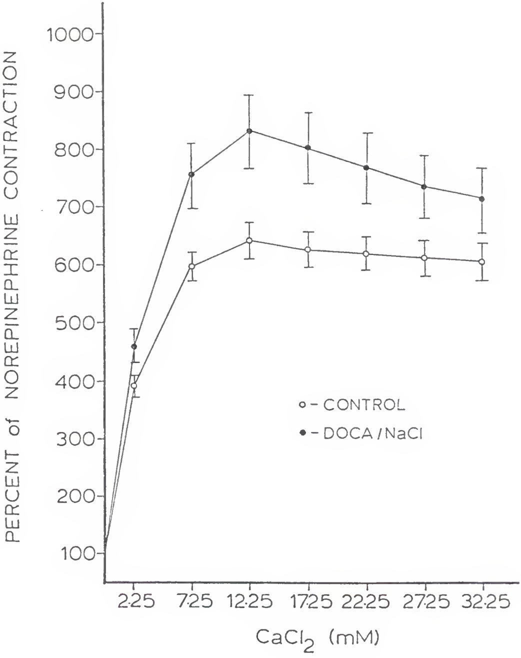

PERCENT of NOREPINEPHRINE CONTRACTION
1000
900
800
700
600
500
400
300
200
100
2·25
7·25
12·25
17·25
22·25
27·25
32·25
$CaCl_2$ (mM)
○ - CONTROL
● - DOCA / NaCl

Figure 37. Dose-response relationship between concentration of $CaCl_2$ and response, expressed as a percent of norepinephrine contraction, of rings of femoral artery from control and DOCA/NaCl hypertensive rats equilibrated in a low calcium (0.25 mM), low potassium (3.0 mM) Krebs physiological solution. A significant difference ($p < .001$) in the response of vascular smooth muscle to increasing concentrations of $CaCl_2$ was observed between the two groups. Each point represents the mean and standard error of 12 rings.

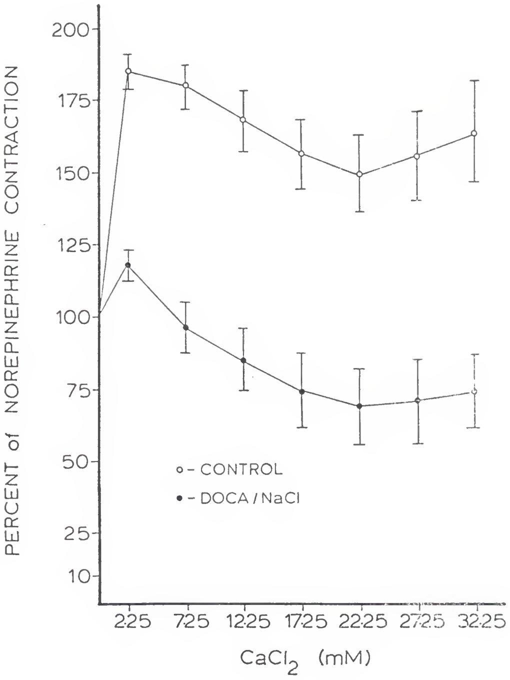
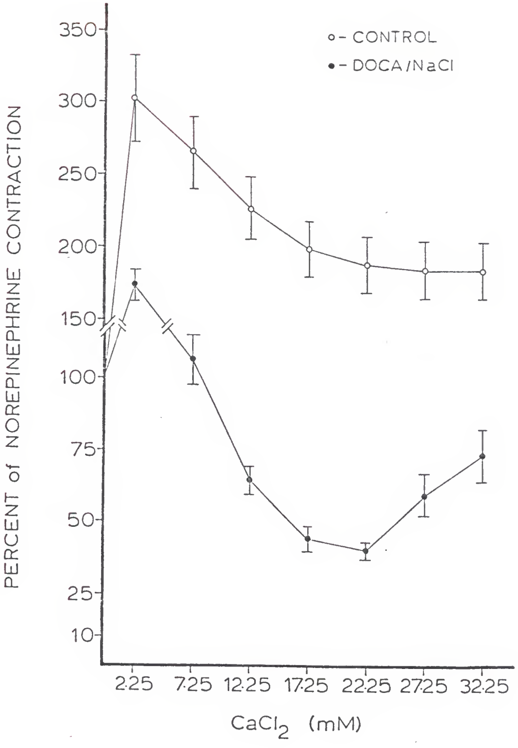

PERCENT of NOREPINEPHRINE CONTRACTION
200
175
150
125
100
75
50
25
10
2·25
7·25
12·25
17·25
22·25
27·25
32·25
CaCl$_2$ (mM)
○ - CONTROL
● - DOCA / NaCl

Figure 38. Dose-response relationship between concentration of $CaCl_2$ and response, expressed as a percent of norepinephrine contraction, of rings of femoral artery from control and DOCA/NaCl hypertensive rats equilibrated in a low calcium (0.25 mM), high potassium (10 mM) Krebs physiological solution. A significant difference ($p < .001$) in the response of vascular smooth muscle to increasing concentrations of $CaCl_2$ was observed between the two groups. Each point represents the mean and standard error of 12 rings.


○ - CONTROL
● - DOCA/NaCl
PERCENT of NOREPINEPHRINE CONTRACTION
350
300
250
200
150
100
75
50
25
10
2·25
7·25
12·25
17·25
22·25
27·25
32·25
$CaCl_2$ (mM)

12.25 mM $CaCl_2$. Expressing the data as a percent of the NE response in 2.25 mM $CaCl_2$ (Figure 39) in the 0 mM potassium Krebs shows an increase in tension in both groups at 7.25 mM and 12.25 mM $CaCl_2$, which was significantly greater in the DOCA/NaCl hypertensive rat. A decrease in tension was seen at doses greater than 12.25 mM $CaCl_2$. This relaxation was greater in femoral smooth muscle from DOCA/NaCl rats when compared to controls (26 and 10 percent, respectively). Dose-response curves to $CaCl_2$ in 3.0 mM and 10 mM potassium Krebs, expressed as a percent of the NE response in 2.25 mM $CaCl_2$, are shown in Figures 40 and 41. A significant increase in the response of femoral smooth muscle from DOCA/NaCl hypertensive rats were observed at all concentrations of $CaCl_2$ in both the low and high potassium Krebs when compared to controls. In 3.0 mM potassium Krebs, maximal relaxation occurred at 22.25 mM $CaCl_2$ for both groups and was 41 percent in the DOCA/NaCl group and 20 percent in controls. Maximal relaxation in 10 mM potassium Krebs also occurred at 22.25 mM $CaCl_2$ in both groups and was 77 percent in the DOCA/NaCl group and 37 percent in controls.

In order to obtain a better appreciation of the effect of extracellular potassium and ouabain on calcium relaxation, the data within each group for each concentration of potassium and ouabain were graphed together. Figure 42 shows the dose-response curve to $CaCl_2$ for VSM from controls in the presence of either ouabain or 0 mM,

Figure 39. Dose-response relationship between concentration of $CaCl_2$ and response, expressed as a percent of norepinephrine contraction in 2.25 mM calcium, of rings of femoral artery from control and DOCA/NaCl hypertensive rats equilibrated in a low calcium (0.25 mM), potassium-free Krebs physiological solution. Each response is calculated from the data in Figure 36 and is shown as a percent of the response at 2.25 mM $CaCl_2$. The response of vascular smooth muscle from DOCA/NaCl hypertensive rats was significantly greater ($p < .05$) than controls at $CaCl_2$ concentrations of 7.25 to 17.25 mM. Each point represents the mean and standard error of 12 rings.

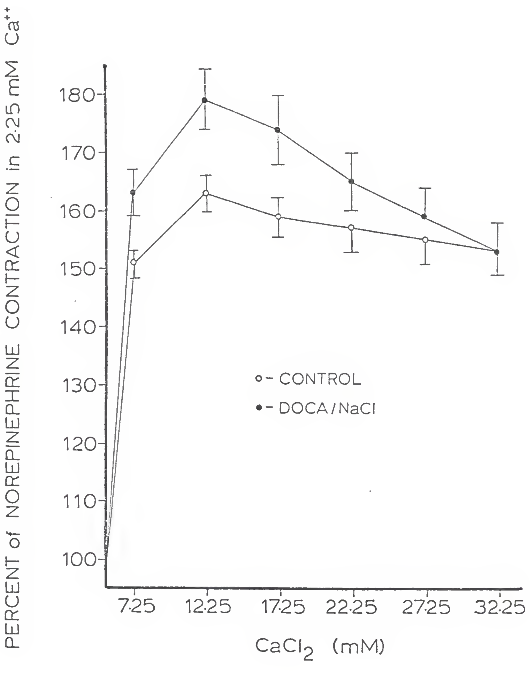

PERCENT of NOREPINEPHRINE CONTRACTION in 2·25 mM Ca++
180
170
160
150
140
130
120
110
100
7·25
12·25
17·25
22·25
27·25
32·25
CaCl2 (mM)
○ - CONTROL
● - DOCA / NaCl

Figure 40. Dose-response relationship between concentration of $CaCl_2$ and relaxation, expressed as a percent of norepinephrine contraction in 2.25 mM calcium, of rings of femoral artery from control and DOCA/NaCl hypertensive rats equilibrated in a low calcium (0.25 mM), low potassium (3.0 mM) Krebs physiological solution. Each response is calculated from the data in Figure 37 and is shown as a percent of the response at 2.25 mM $CaCl_2$. Calcium-induced relaxation of vascular smooth muscle from DOCA/NaCl hypertensive rats was significantly greater ($p < .05$) than controls. Each point represents the mean and standard error of 12 rings.

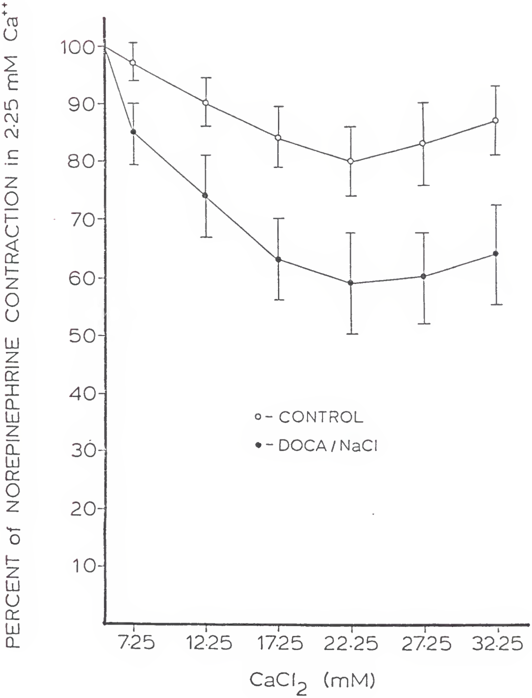

PERCENT of NOREPINEPHRINE CONTRACTION in 2.25 mM Ca^{++}
100
90
80
70
60
50
40
30
20
10
7.25
12.25
17.25
22.25
27.25
32.25
$CaCl_2$ (mM)
○ - CONTROL
● - DOCA / NaCl

Figure 41. Dose-response relationship between concentration of $CaCl_2$ and relaxation, expressed as a percent of norepinephrine contraction in 2.25 mM calcium, of rings of femoral artery from control and DOCA/NaCl hypertensive rats equilibrated in a low calcium (0.25 mM), high potassium (10 mM) Krebs physiological solution. Each response is calculated from the data in Figure 38 and is shown as a percent of the response at 2.25 mM $CaCl_2$. Calcium-induced relaxation of vascular smooth muscle from DOCA/NaCl hypertensive rats was significantly greater ($p < .001$) than controls. Each point represents the mean and standard error of 12 rings.

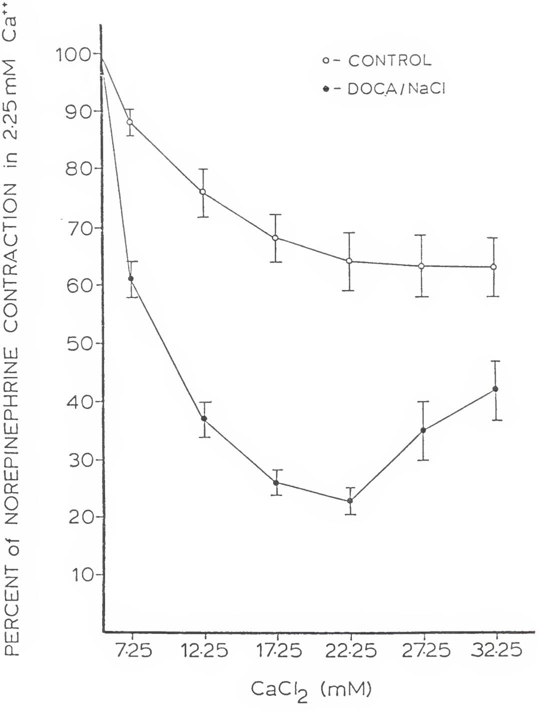
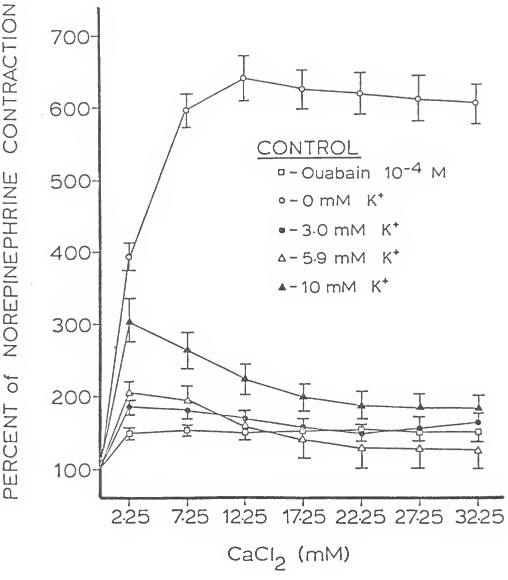

PERCENT of NOREPINEPHRINE CONTRACTION in 2·25 mM Ca⁺⁺
100
90
80
70
60
50
40
30
20
10
○ - CONTROL
● - DOCA/NaCl
7·25
12·25
17·25
22·25
27·25
32·25
CaCl₂ (mM)

Figure 42. Dose-response relationship between concentration of $CaCl_2$ and response, expressed as a percent of norepinephrine contraction, of rings of femoral artery from control rats equilibrated in a low calcium (0.25 mM) Krebs physiological solution in the presence of either ouabain (10^{-4} M), 0 mM, 3.0 mM, 5.9 mM, or 10 mM potassium. The response of vascular smooth muscle to increasing concentrations of $CaCl_2$ in 0 mM potassium was significantly different ($p < .001$) from all other groups. At $CaCl_2$ concentrations of 2.25 to 17.25 mM the response in 10 mM potassium was significantly greater ($p < .05$) than that in ouabain, 3.0, of 5.9 mM potassium. Each point represents the mean and standard error of 12 rings.


PERCENT of NOREPINEPHRINE CONTRACTION
700
600
500
400
300
200
100
2·25
7·25
12·25
17·25
22·25
27·25
32·25
CaCl₂ (mM)
CONTROL
□ - Ouabain 10⁻⁴ M
○ - 0 mM K⁺
● - 3·0 mM K⁺
△ - 5·9 mM K⁺
▲ - 10 mM K⁺

3.0 mM, 5.9 mM or 10 mM potassium. An increase in tension of greater than 500 percent was observed in 0 mM potassium. At the other levels of potassium a concentration-dependent increase in tension was observed at 2.25 mM $CaCl_2$. The greatest response occurred in 10 mM potassium (200 percent increase) and the lowest response in 3.0 mM potassium (80 percent increase). In the presence of ouabain, a slight increase in tension (40 percent) occurred at 2.25 mM $CaCl_2$. A slight decrease in tension occurred in 0 mM potassium at $CaCl_2$ concentrations greater than 12.25 mM, while no alteration in tension occurred in the presence of ouabain. A slight decrease in tension occurred with increasing concentration of $CaCl_2$ in 3.0 mM potassium. $CaCl_2$-induced relaxation was similar in both 5.9 mM and 10 mM potassium. Expressing the data as a percent of the response at 2.25 mM $CaCl_2$ emphasizes the relaxant properties of Ca^{++} (Figure 43). Maximal relaxation in 5.9 mM potassium was approximately 40 percent and occurred at 32.25 mM $CaCl_2$. Incubation in 3.0 mM potassium attenuated the response to $CaCl_2$ with only a 20 percent maximal relaxation. No increase in the response to $CaCl_2$ was observed in 10 mM potassium. Ouabain and 0 mM potassium prevented the relaxation induced by $CaCl_2$.

The response of VSM from DOCA/NaCl hypertensive rats to $CaCl_2$ was similar to controls in the presence of ouabain (50 percent increase in tension) and 0 mM potassium (600 percent increase in tension (Figure 44).

Figure 43. Dose-response relationship between concentration of $CaCl_2$ and relaxation, expressed as a percent of norepinephrine contraction in 2.25 mM calcium, of rings of femoral artery from control rats equilibrated in a low calcium (0.25 mM) Krebs physiological solution in the presence of either ouabain (10^{-4} M), 0 mM, 3.0 mM, 5.9 mM, of 10 mM potassium. Each response is calculated from the data in Figure 42 and is shown as a percent of the response at 2.25 mM $CaCl_2$. Ouabain and 0 mM potassium prevented the calcium-induced relaxation of vascular smooth muscle. Maximal calcium-induced relaxation occurred in the presence of 5.9 and 10 mM potassium. A significant decrease ($p < .05$) in the response to $CaCl_2$ was observed in the presence of 3.0 mM potassium. Each point represents the mean and standard error of 12 rings.

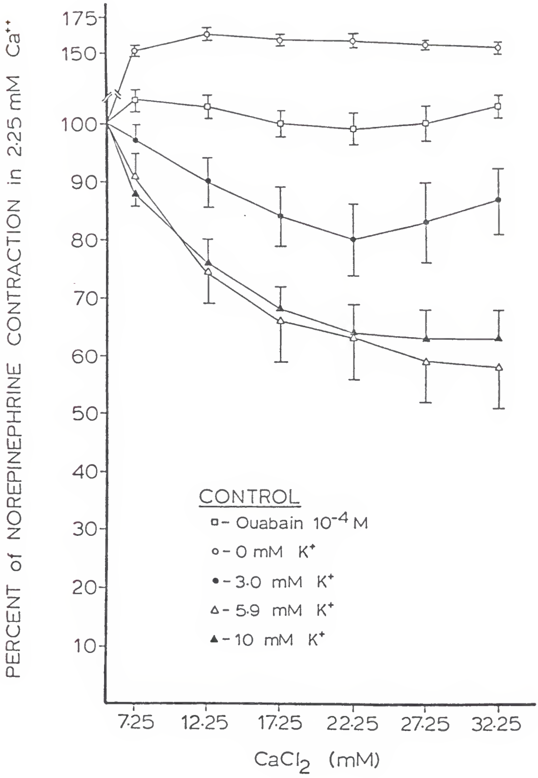
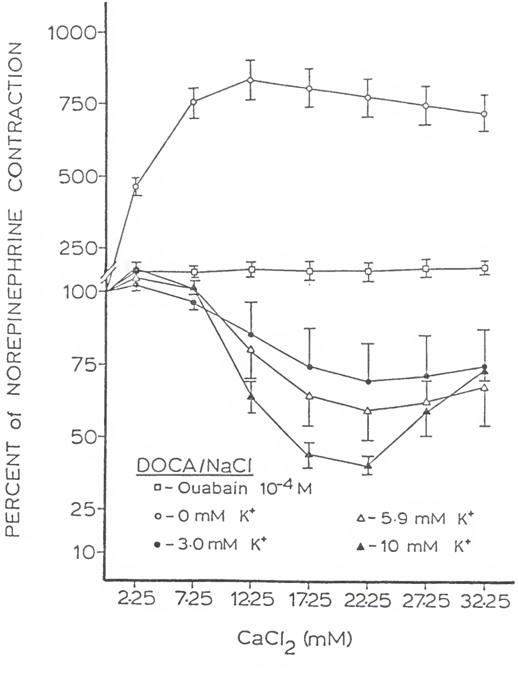

PERCENT of NOREPINEPHRINE CONTRACTION in 2·25 mM Ca⁺⁺
175
150
100
90
80
70
60
50
40
30
20
10
7·25
12·25
17·25
22·25
27·25
32·25
CaCl₂ (mM)
CONTROL
□ - Ouabain 10⁻⁴ M
○ - 0 mM K⁺
● - 3·0 mM K⁺
△ - 5·9 mM K⁺
▲ - 10 mM K⁺

Figure 44. Dose-response relationship between concentration of $CaCl_2$ and response, expressed as a percent of norepinephrine contraction, of rings of femoral artery from DOCA/NaCl hypertensive rats equilibrated in a low calcium (0.25 mM) Krebs physiological solution in the presence of either ouabain (10^{-4} M), 0 mM, 3.0 mM, 5.9 mM, or 10 mM potassium. The response of vascular smooth muscle to increasing concentrations of $CaCl_2$ in 0 mM potassium, as well as ouabain, was significantly different ($p < .001$) from all other groups. The response in 10 mM potassium was significantly different ($p < .05$) from the response in both 5.9 mM and 3.0 mM potassium at $CaCl_2$ concentrations of 12.25 to 22.25 mM. Each point represents the mean and standard error of 12 rings.


PERCENT of NOREPINEPHRINE CONTRACTION
1000
750
500
250
100
75
50
25
10
2.25
7.25
12.25
17.25
22.25
27.25
32.25
CaCl$_2$ (mM)
DOCA/NaCl
□ - Ouabain 10^{-4} M
○ - 0 mM K$^+$
● - 3.0 mM K$^+$
△ - 5.9 mM K$^+$
▲ - 10 mM K$^+$

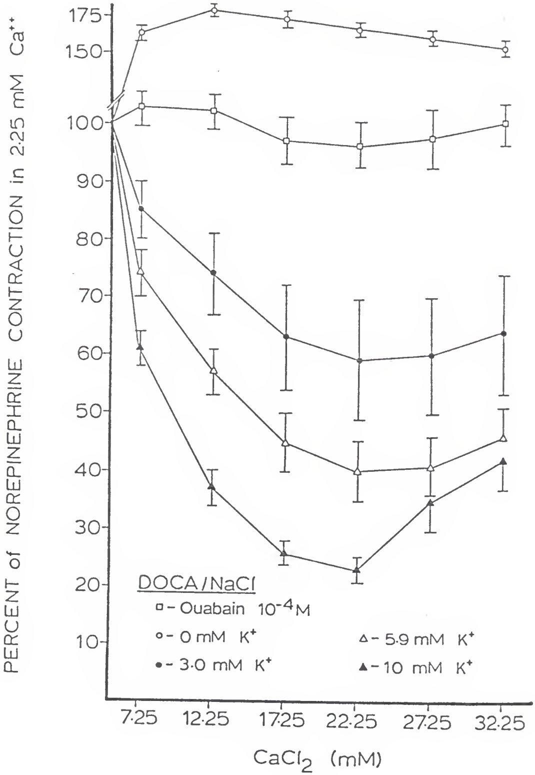
The response in 3.0 mM, 5.9 mM, and 10 mM potassium, however, was different. All 3 groups responded in a similar fashion to 2.25 mM and 7.25 mM $CaCl_2$ and maximal relaxation occurred at 22.25 mM $CaCl_2$. However, a potassium concentration-dependent response was observed, with the greatest response occurring in 10 mM potassium and the lowest in 3.0 mM. These differences are further emphasized when the data are expressed as a percent of the response at 2.25 mM $CaCl_2$ (Figure 45). Again, 0 mM potassium and ouabain blocked the relaxation response to $CaCl_2$. The response in 10 mM potassium was significantly greater at $CaCl_2$ concentrations of 7.25 to 22.25 mM when compared to 5.9 mM potassium. Incubation in 3.0 mM potassium resulted in a significant decrease in the response to accumulative addition of $CaCl_2$. The maximal response in 3.0 mM, 5.9 mM, and 10 mM potassium were 40, 60, and 78 percent, respectively.

Discussion

It is evident from the results of this study that sodium-potassium pump activity is increased in the DOCA/NaCl treated rat.

Potassium-relaxation is one method commonly used to measure sodium-potassium pump activity in VSM. This technique has been adapted for both in vivo and in vitro preparations, however, it appears that this method has limitations since conflicting results have been obtained.

Figure 45. Dose-response relationship between concentration of $CaCl_2$ and relaxation, expressed as a percent of norepinephrine contraction in 2.25 mM calcium, of rings of femoral artery from DOCA/NaCl hypertensive rats equilibrated in a low calcium (0.25 mM) Krebs physiological solution in the presence of either ouabain (10^{-4} M), 0 mM, 3.0 mM, 5.9 mM, or 10 mM potassium. Each response is calculated from the data in Figure 44 and is shown as a percent of the response at 2.25 mM $CaCl_2$. Ouabain and 0 mM potassium prevented the calcium-induced relaxation of vascular smooth muscle. A significant ($p < .05$) potassium concentration-dependent response was observed, with the greatest relaxation occurring in 10 mM potassium. Each point represents the mean and standard error of 12 rings.


PERCENT of NOREPINEPHRINE CONTRACTION in 2·25 mM Ca⁺⁺
175
150
100
90
80
70
60
50
40
30
20
10
7·25
12·25
17·25
22·25
27·25
32·25
CaCl2 (mM)
DOCA/NaCl
□ - Ouabain 10⁻⁴M
○ - 0 mM K⁺
● - 3.0 mM K⁺
△ - 5.9 mM K⁺
▲ - 10 mM K⁺

It appears that the response to potassium varies with several parameters, such as length of incubation time and concentration of potassium added to the bath (Webb et al., 1981a; Webb et al., 1983a). The relationship between extracellular potassium concentration in the bath during the incubation period and the magnitude of relaxation is sigmoidal in nature, with a 70 percent relaxation occurring in 0.5 mM potassium and only 10 percent in 2.0 mM potassium. A concentration of 0.6 mM potassium was chosen for the present study since this concentration coincided with a part of the sigmoidal curve in which an alteration of sodium-potassium pump activity would be most apparent. In addition, the response of VSM which has been incubated for a period of time in potassium-free physiological solution can be greatly depressed.

A dose-response curve to potassium was generated beginning at low concentrations up to approximately physiological levels. This was done to give a better indication of the activity of the sodium-potassium pump in the physiological range. It was apparent from the results that sodium-potassium pump activity in VSM from controls shows maximal pump stimulation (hypothetical) around normal physiological levels (Figure 33). On the other hand, VSM from the DOCA/NaCl hypertensive rat have a much higher activity in normal conditions and could be considered to have normal sodium-potassium pump activity in the range of 1.4 to 2.0 mM potassium. At concentrations of potassium less than 2.0 mM, controls have more than a 50 percent reduction in pump activity. This suggests that the DOCA/NaCl rat has a large reserve in terms of pump activity.

Webb and Bohr (1978) suggested that membrane stabilization or relaxation of VSM induced by calcium was mediated through the sodium-potassium pump. Therefore, this author felt that this effect of calcium could be used as an index of pump activity.

Dose-response curves to calcium were carried out at various levels of pump activity: inhibition with ouabain or potassium-free Krebs; low pump activity with 3.0 mM potassium Krebs; normal pump activity with 5.9 mM potassium Krebs; and high pump activity with 10 mM potassium Krebs. It was evident that calcium relaxation is mediated by the sodium-potassium pump since ouabain and potassium-free Krebs blocked the relaxation. In addition, the magnitude of relaxation was dependent upon the level of extracellular potassium, which is important in regulating pump activity.

There is one observation, however, that needs to be explained. The response of VSM to calcium in the presence of ouabain and potassium-free Krebs would be expected to be similar. However, this is not the case. The response to calcium was much more exaggerated in the potassium-free than in the presence of ouabain. This can be explained by the methods used. When potassium-free

Krebs was used, the tissues were incubated in it for 90 minutes. When ouabain was used, it was added only a few minutes prior to the dose-response curve. During the 90 minute equilibration period in potassium-free Krebs, the VSM cells became loaded with sodium due to pump inhibition and, therefore, when calcium was added, the sodium-calcium exchange was augmented and the response of the VSM was greatly exaggerated.

The results of the potassium relaxation experiment are supported by the findings in the calcium relaxation experiment. At 3.0 mM extracellular potassium, maximal relaxation of VSM from controls in response to calcium was decreased when compared to response in the normal physiological solution (5.9 mM potassium)(Figure 43). This supports the observation in the potassium relaxation experiment in that VSM from controls relaxed only 80 percent at a level of 3.0 mM potassium.

It was also stated that maximal relaxation (and, hypothetically, maximal pump activity) in response to potassium most likely occurs around normal physiological levels. This is reflected, again, in the calcium relaxation experiment (Figure 43). The response to calcium in 10 mM potassium was no greater than in 5.9 mM for VSM from controls. This suggests that maximal pump activity in VSM from controls occurs in the normal physiological range.

The response of VSM from DOCA/NaCl hypertensive rats, however, is much greater. As mentioned earlier, potassium concentrations in a range of 1.5 to 2.0 mM appear to result in sodium-potassium pump activity equivalent to controls in normal potassium. Therefore, at higher levels of potassium, the pump activity in DOCA/NaCl rats may be even higher. Figure 45 from the calcium relaxation experiment shows that VSM from DOCA/NaCl hypertensive rats respond with a greater magnitude of relaxation to calcium as the extracellular potassium is increased to 10 mM. It should also be noted that the response of VSM from DOCA/NaCl treated rats to calcium in 3.0 mM potassium is similar to VSM from controls in 5.9 mM potassium. This further emphasizes the increase in pump activity of VSM from the DOCA/NaCl rat.

The third experiment in this section confirms the results of the other two in that ouabain-sensitive uptake of ^{86}Rb was increased in the DOCA/NaCl hypertensive rat. A time course of uptake was done to determine if any alterations in the rate of ^{86}Rb uptake occur. There was a significant increase in uptake at all time periods in the DOCA/NaCl rat (Table 3). It is interesting to note that uptake per minute is fairly constant in controls, whereas in the DOCA/NaCl rats the rate is quite high for the first 3 minutes and decreases progressively toward control values with increasing time intervals of incubation. This is most likely due to a saturation of the uptake system since controls exhibited a decrease in rate at 20 minutes, also.

However, this increase in rate emphasizes the large increase in pump activity in VSM from DOCA/NaCl rats.

Ouabain-insensitive uptake was similar in both groups suggesting that no alterations exist in passive ion movement. This is in opposition to the findings of Friedman et al. (1975) who suggested that the increase in pump activity is a result of increases in membrane ion permeability.

The results of these experiments agree with the findings of Webb (1982b) and Brock et al. (1982) that an increase in sodium-potassium pump activity occurs in DOCA/NaCl hypertensive rats. They are in conflict with Pamnani et al. (1980a, 1980b), however. It has been suggested that these differences are due to differences in experimental design. The length of preincubation may allow for the washout of a ouabain-like humoral factor. If uptake analysis is performed immediately after excising the vessel, a decrease in pump activity is seen (Pamnani et al., 1981b). However, if the vessel is incubated for a longer period of time, this factor may be washed out and an increase in pump activity is observed. The increase in pump activity may be due to an actual increase in the number of pump sites as a result of a compensatory mechanism in opposition to the humoral factor.

In summary, the results of these three experiments clearly show that sodium-potassium pump activity is increased in VSM from DOCA/NaCl hypertensive rats. In addition, the membrane stabilizing effect of calcium appears to be useful as an index of quantifying pump activity since it was potassium concentration-dependent and agreed with the results of the other two experiments in that sodium-potassium pump activity was increased in the DOCA/NaCl rat.

PART V
SODIUM-POTASSIUM PUMP AND VASCULAR REACTIVITY IN DOCA/NaCl HYPERTENSIVE RATS

Introduction

Although the role of action potentials in the activation of skeletal and cardiac muscle is well established, such a role for electromechanical coupling in smooth muscle, and in particular VSM, is less certain (Johansson and Somlyo, 1980). Su et al. (1964) first reported that NE applied exogenously to rabbit pulmonary artery produced a contraction without a change in membrane potential. A later study (Somlyo and Somlyo, 1968) using the same vessel, however, showed that NE does depolarize the VSM cells, but that a contraction also could be generated by nonelectrical means. More recently, Trapani et al. (1981) and Mulvany et al. (1982) have shown that variations in membrane potential occur in smaller resistance vessels upon stimulation by exogenous NE. They suggest that these changes in membrane potential have an important modulating influence on tension development in response to NE.

Skaug and Detar (1981a) have suggested that vascular reactivity to NE is dependent upon two opposing fundamental properties of VSM; contractility and electrogenesis (Na, K-ATPase). Contractility is defined as the positive component to overall reactivity while electrogenesis is the negative component. As an example, an increase in reactivity of VSM could occur with either an increase in contractility or a decrease in electrogenesis. Further studies have shown that altering extracellular potassium ion concentration can change the contractile properties of VSM to NE and that this effect is mediated through the sodium-potassium pump (Skaug and Detar, 1981b).

It appears, then, that the sodium-potassium pump and its effect on membrane potential in VSM may play an important role in regulating α-adrenergic mediated responses. Broekaert and Godfraind (1973) showed an increase in the response of rabbit arteries and guinea pig aorta to NE in the presence of ouabain. Similar results were obtained in rat mesenteric resistance vessels using ouabain and potassium-free physiological solutions (Mulvany _et al_., 1982b). However, incubation for prolonged periods in the presence of ouabain resulted in a depressive effect on NE responsiveness.

Since alterations in the sodium-potassium pump occur in VSM from hypertensive animals, it may well be that changes in NE responsiveness are due, in part, to an effect of the sodium-potassium pump. Gothberg _et al_. (1980) showed that the presence of ouabain resulted in a shift to the left of the dose-response curve for norepinephrine which was slightly greater in the SHR than in the WKY rat. Mulvany _et al_. (1980c) observed that mesenteric vessels from SHR showed a greater norepinephrine-activated calcium sensitivity than vessels from WKY. However, the response was reduced in the presence of ouabain so that it was similar to that of the WKY. Recently, Nilsson and Mulvany (1981) have suggested that ouabain is merely blocking the norepinephrine-activated calcium channels and not having its effect through the sodium pump.

Hermsmeyer (1976) and Hermsmeyer et al. (1982) have shown that the membrane potential of VSM cells of the caudal artery from SHR is decreased and suggest that the increased sensitivity of arterial smooth muscle to NE is due to this alteration. Alternatively, caudal arteries from DOCA-hypertensive and Dahl genetic hypertensive rats showed no alteration in membrane potential and furthermore did not show an increase in NE sensitivity. This suggests another mechanism (i.e. a circulating ouabain-like factor or an alteration in calcium metabolism) by which an increase in peripheral vascular resistance is achieved.

As with its effects on norepinephrine-stimulated contraction, ouabain also appears to have an effect on β-receptor mediated actions. Broekaert and Godfraind (1973) showed that ouabain at 10^{-9} M induced a potentiation of the relaxation evoked by β-receptor stimulation with isoproterenol in guinea pig aorta. However, higher doses of ouabain induced dose-dependent reductions in the relaxant effect of isoproterenol. Webb and Bohr (1981) have observed similar responses with rat tail artery strips in that increasing ouabain concentration from 10^{-8} to 10^{-3} M results in a dose-dependent inhibition of the isoproterenol-induced relaxation. They suggested that the pump is necessary for relaxation in response to isoproterenol, however, it is not the primary mechanism by which the relaxation occurs. No studies have been reported on the role of the sodium-potassium pump in alterations in VSM β-adrenergic responsiveness of hypertensive animals.

It is quite evident from the above discussion that little work has been done concerning the interaction of the vascular sodium-potassium pump and adrenergic responsiveness in hypertensive, as well as normotensive, animals. It appears, though, that the sodium-potassium pump does indeed play a role in regulating vascular adrenergic responsiveness. Since responses to adrenergic stimuli are altered in the hypertensive state along with apparent changes in the sodium-potassium pump, it is quite possible that the sodium-potassium pump is, to some extent, regulating adrenergic responsiveness. Therefore, in this part of the dissertation, experiments will be reported which determined the importance of the sodium-potassium pump in mediating vascular adrenergic responsiveness in both control and DOCA/NaCl hypertensive rats.

Dose-response curves to ISO and NE were carried out under conditions in which the activity of the sodium-potassium pump was either maximally inhibited (potassium-free Krebs physiological solution), maximally stimulated

(10 mM potassium Krebs physiological solution)(Anderson, 1976; Skaug and Detar, 1981b), or decreased by 50 percent (temperature of the muscle bath at 27° C). It is well established that a decrease in sodium-potassium pump activity occurs with a decrease in temperature (Schwartz *et al*., 1975). In addition, Webb and Bohr (1978) have shown an inhibition of potassium-induced relaxation (a measure of sodium-potassium pump activity) of VSM by cooling to 15° C. A decrease in the temperature by 10° C (from 37° C to 27° C) is known to decrease the activity of the sodium-potassium pump by approximately 50 percent.

Experiment VII

This experiment was designed to investigate the role of Na,K-ATPase (sodium-potassium pump) in the alterations that occur in vascular reactivity of the DOCA/NaCl hypertensive rat.

Methods

Part A

Twelve 6 week-old male S-D rats were used in this experiment. DOCA/NaCl hypertension was induced in 6 of these rats as described in General Methods. At 10 weeks of age (4 weeks postimplant) all animals were sacrificed and rings of femoral artery prepared. After the 90 minute equilibration period in a normal Krebs physiological solution at the appropriate preload force, tissues were maximally stimulated with serotonin (10^{-7} M). The response was allowed to stabilize (100 percent value) after which accumulative dose-response curves to ISO and $NaNO_2$ were generated. Relaxation in response to ISO and $NaNO_2$ were expressed as a percent of the serotonin contraction.

Part B

Twenty-four 6 week-old male S-D rats were used in this experiment. DOCA/NaCl hypertension was induced in 12 of these rats as described in General Methods. At 10 weeks of age (4 weeks postimplant) all animals were sacrificed and rings of femoral artery prepared. The tissues were equilibrated for 90 minutes in a normal Krebs physiological solution at the appropriate preload force, after which a dose-response curve to KCl was generated. Fifteen minutes prior to and during the ISO, $NaNO_2$ and NE dose-response curves, the tissues were incubated in either a potassium-free (0 mM) or high potassium (10 mM) Krebs solution. Dose-response curves to ISO and $NaNO_2$ were carried out following a maximal contraction with 10^{-7} M serotonin. During the one hour wash period prior to the NE dose-response curve, tissues were incubated in the normal Krebs solution.

Part C

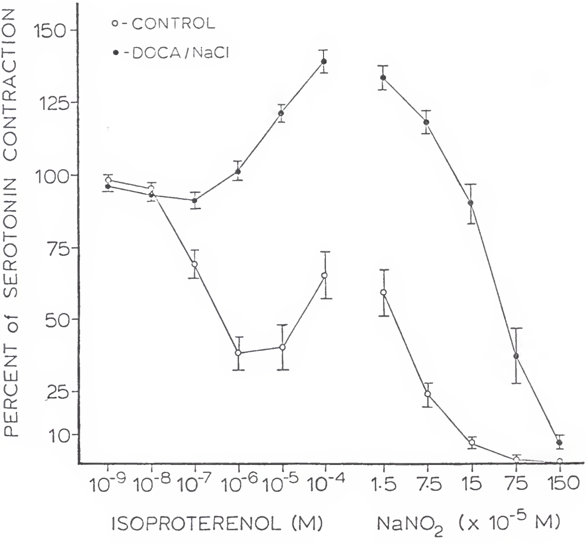
Twelve 6 week-old male S-D rats were used in this experiment. DOCA/NaCl hypertension was induced in 6 of these rats as described in General Methods. At 10 weeks of age (4 weeks postimplant) all animals were sacrificed and rings of femoral artery prepared. The 90 minute equilibration period and dose-response curves to KCl, ISO, $NaNO_2$, and NE were carried out in a normal Krebs physiological solution at 27° C. As with Parts A and B, ISO and $NaNO_2$ dose-response curves were carried out following a maximal contraction with 10^{-7} M serotonin.

Results

Part A

A significant decrease in the response of femoral smooth muscle from DOCA/NaCl hypertensive rats to accumulative addition of ISO was observed (Figure 46). Both groups showed an initial response at 10^{-9} M ISO with DOCA/NaCl rats relaxing 4 percent and controls relaxing 2 percent. Maximal relaxation occurred at 10^{-7} M ISO in the DOCA/NaCl group (9 percent relaxation) and at 10^{-6} M ISO in controls (62 percent relaxation). An increase in tension occurred at concentrations of 10^{-6} M to 10^{-4} M ISO in DOCA/NaCl hypertensive rats and at 10^{-5} to 10^{-4} M in controls. The maximum response was greater in the DOCA/NaCl group (48 percent increase in tension) when compared to controls (27 percent increase in tension). A significant difference in the response to low concentrations of $NaNO_2$ was observed between the two groups which is a result of the difference at 10^{-4} M ISO. Maximal relaxation in response to $NaNO_2$ was similar for both groups (99 percent in controls and 93 percent in DOCA/NaCl rats).

Figure 46. Dose-response relationship between concentration of isoproterenol and sodium nitrite and relaxation, expressed as a percent of serotonin contraction, of rings of femoral artery from control and DOCA/NaCl hypertensive rats. Maximum isoproterenol-induced relaxation was significantly attenuated ($p < .001$) in vascular smooth muscle from DOCA/NaCl hypertensive rats. No difference in the maximum response to sodium nitrite was observed. Each point represents the mean and standard error of 12 rings.


PERCENT of SEROTONIN CONTRACTION
150
125
100
75
50
25
10
○ - CONTROL
● - DOCA / NaCl
10^-9 10^-8 10^-7 10^-6 10^-5 10^-4
1·5 7·5 15 75 150
ISOPROTERENOL (M)
NaNO2 (x 10^-5 M)

Part B

ISO-induced relaxation in 0 mM potassium Krebs was significantly attenuated in rings of femoral artery from DOCA/NaCl hypertensive rats (Figure 47). Controls responded initially at 10^{-9} M ISO (4 percent relaxation) with maximal relaxation occurring at 10^{-5} M (69 percent relaxation). The initial response of femoral smooth muscle from the DOCA/NaCl group occurred at 10^{-8} M ISO (3 percent relaxation) with a maximal response occurring at 10^{-4} M (34 percent relaxation). No increase in tension was observed in DOCA/NaCl rats while controls showed only a slight response (1 percent increase in tension) at 10^{-4} m ISO. Maximal relaxation in 0 mM potassium in response to $NaNO_2$ was similar in both groups (100 percent for controls and 98 percent for DOCA/NaCl). The difference at low concentrations is due to the difference at 10^{-4} M ISO.

Development of active tension by rings of femoral artery from control and DOCA/NaCl hypertensive rats in response to accumulative addition of NE in 0 mM potassium Krebs is shown in Figure 48. A significant increase in the NE sensitivity of femoral smooth muscle from DOCA/NaCl rats was observed. However, a slight decrease in the contractility was seen when compared to controls. Expressing the data as a percent of the maximal NE response (Figure 49) further emphasizes the increase in NE sensitivity of femoral smooth muscle from DOCA/NaCl rats in 0 mM potassium

Figure 47. Dose-response relationship between concentration of isoproterenol and sodium nitrite and relaxation, expressed as a percent of serotonin contraction, of rings of femoral artery from control and DOCA/NaCl hypertensive rats in potassium-free Krebs physiological solution. Maximum isoproterenol-induced relaxation was significantly attenuated ($p < .001$) in vascular smooth muscle from DOCA/NaCl hypertensive rats when compared to controls. No difference in the maximum response to sodium nitrite was observed. Each point represents the mean and standard error of 12 rings.

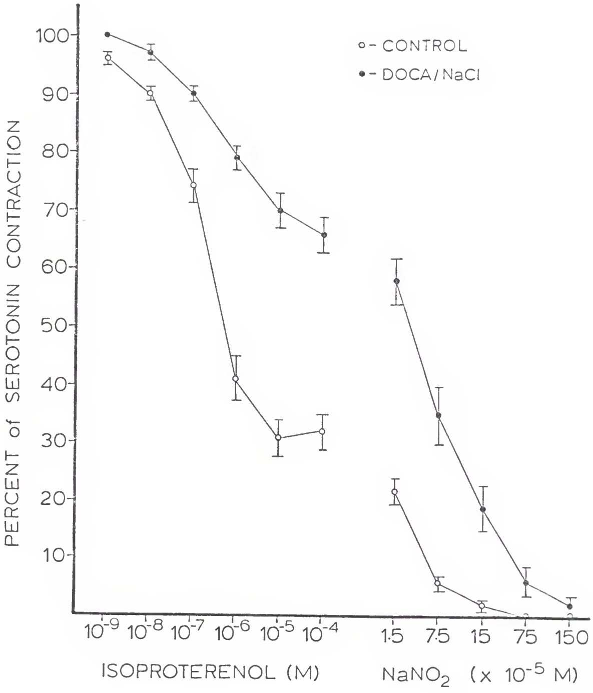

○ - CONTROL
● - DOCA/NaCl
PERCENT of SEROTONIN CONTRACTION
100
90
80
70
60
50
40
30
20
10
10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5} 10^{-4}
ISOPROTERENOL (M)
1·5 7·5 15 75 150
$NaNO_2$ (x 10^{-5} M)

Figure 48. Dose-response relationship between concentration of norepinephrine and development of active tension by rings of femoral artery from control and DOCA/NaCl hypertensive rats in potassium-free Krebs physiological solution. A significant increase ($p < .001$) in the norepinephrine sensitivity (10^{-10} to 10^{-9} M) of vascular smooth muscle from DOCA/NaCl hypertensive rats was seen. Each point represents the mean and standard error of 12 rings.

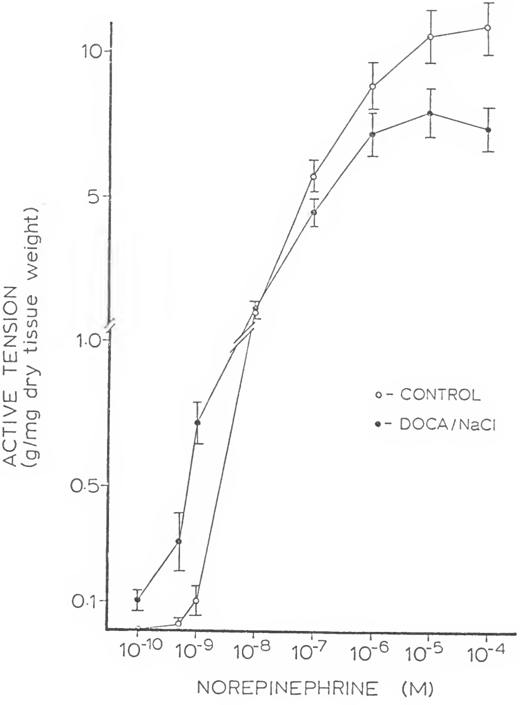
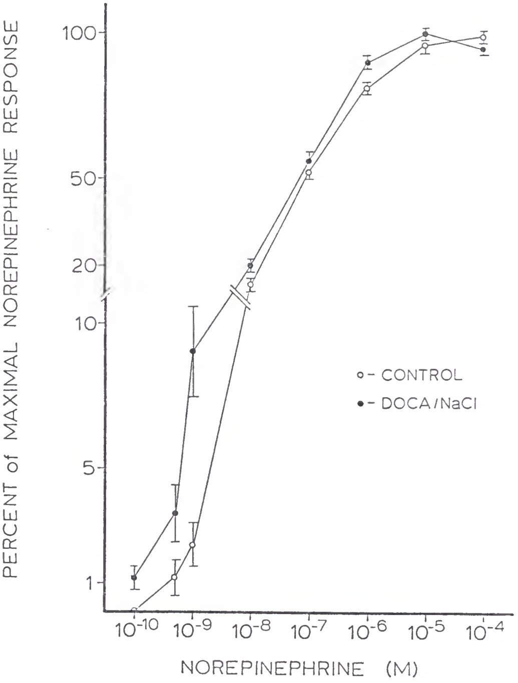

ACTIVE TENSION
(g/mg dry tissue weight)
10
5
1.0
0.5
0.1
10-10
10-9
10-8
10-7
10-6
10-5
10-4
NOREPINEPHRINE (M)
○ - CONTROL
● - DOCA/NaCl

Figure 49. Dose-response relationship between concentration of norepinephrine and percent of maximal norepinephrine response of rings of femoral artery from control and DOCA/NaCl hypertensive rats in potassium-free Krebs physiological solution. Each response is calculated from the data in Figure 48 and is shown as a percent of the maximum active tension generated. A significant increase ($p < .05$ to $p \leq .001$) in the norepinephrine sensitivity (10^{-10} to 10^{-8} M) of vascular smooth muscle from DOCA/NaCl hypertensive rats was seen. Each point represents the mean and standard error of 12 rings.


PERCENT of MAXIMAL NOREPINEPHRINE RESPONSE
100
50
20
10
5
1
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
NOREPINEPHRINE (M)
○ - CONTROL
● - DOCA/NaCl

Krebs. Effective dose values for control and DOCA/NaCl hypertensive rats were $4.9 \times 10^{-9} \pm 1.0 \times 10^{-9}$ M and $1.25 \times 10^{-9} \pm 0.9 \times 10^{-9}$ M, respectively.

Relaxation of rings of femoral artery from control and DOCA/NaCl hypertensive rats in response to accumulative addition of ISO in 10 mM potassium Krebs is shown in Figure 50. Isoproterenol-induced relaxation was significantly attenuated in DOCA/NaCl hypertensive rats when compared to controls. Femoral smooth muscle from the DOCA/NaCl group responded initially at 10^{-8} M ISO (1 percent relaxation) with maximal relaxation occurring at 10^{-6} M (5 percent relaxation). An increase in tension was observed at 10^{-5} M (13 percent) and 10^{-4} M (23 percent). The initial response to ISO in controls occurred at 10^{-9} M (2 percent relaxation) with maximal relaxation (69 percent) occurring at 10^{-6} and 10^{-5} M. A 23 percent increase in tension was observed at 10^{-4} M ISO. Sodium nitrite-induced relaxation was significantly different between the two groups at low concentrations, however, maximal relaxation was similar (100 percent in controls and 93 percent in DOCA/NaCl).

Dose-response curves of rings of femoral artery from control and DOCA/NaCl hypertensive rats to accumulative addition of NE in 10 mM potassium Krebs is shown in Figure 51. A significant increase in both NE sensitivity and contractility was observed in femoral smooth muscle from DOCA/NaCl hypertensive rats. When the data are expressed as a percent of the maximal NE response (Figure

Figure 50. Dose-response relationship between concentration of isoproterenol and sodium nitrite and relaxation, expressed as a percent of serotonin contraction, of rings of femoral artery from control and DOCA/NaCl hypertensive rats in high potassium (10 mM) Krebs physiological solution. Maximum isoproterenol-induced relaxation was significantly attenuated ($p < .001$) in vascular smooth muscle from DOCA/NaCl hypertensive rats when compared to controls. No difference in the maximum response to sodium nitrite was observed. Each point represents the mean and standard error of 12 rings.

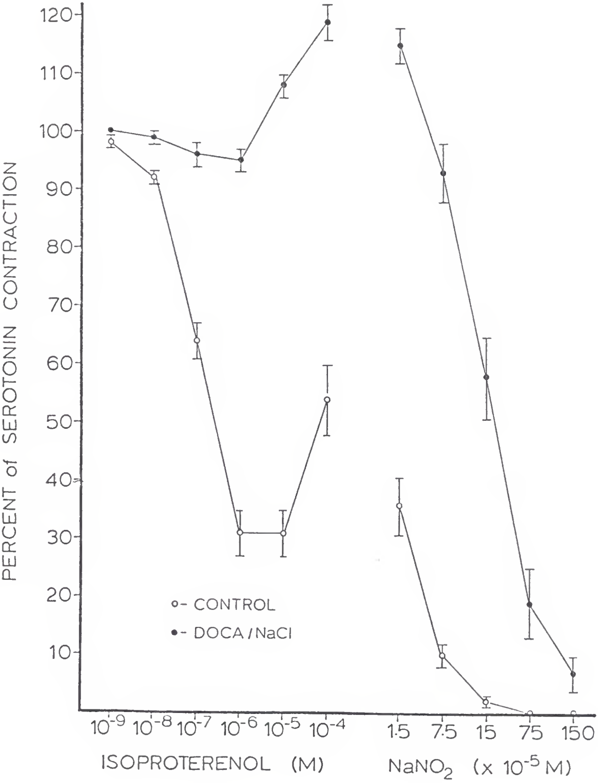
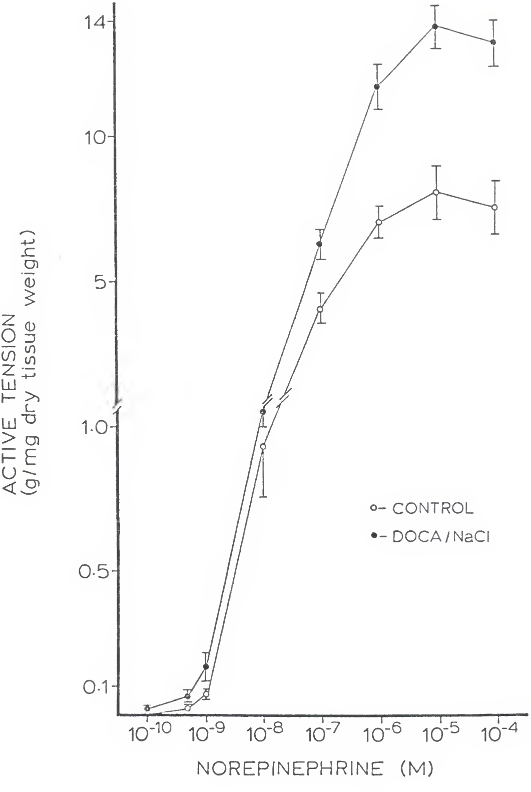

PERCENT of SEROTONIN CONTRACTION
120
110
100
90
80
70
60
50
40
30
20
10
o- CONTROL
•- DOCA / NaCl
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
1·5
7·5
15
75
150
ISOPROTERENOL (M)
$NaNO_2$ (x 10^{-5} M)

Figure 51. Dose-response relationship between concentration of norepinephrine and development of active tension by rings of femoral artery from control and DOCA/NaCl hypertensive rats in high potassium (10 mM) Krebs physiological solution. Norepinephrine sensitivity (10^{-10} to 10^{-9} M and contractility (10^{-6} to 10^{-4} M) were significantly greater ($p < .05$) in vascular smooth muscle from DOCA/NaCl hypertensive rats. Each point represents the mean and standard error of 12 rings.


ACTIVE TENSION
(g/mg dry tissue weight)
14
10
5
1.0
0.5
0.1
10^-10
10^-9
10^-8
10^-7
10^-6
10^-5
10^-4
NOREPINEPHRINE (M)
○- CONTROL
●- DOCA / NaCl

52) no difference between controls and DOCA/NaCl hypertensive rats was observed. ED10 values for control and DOCA/NaCl hypertensive rats were $1.05 \times 10^{-8} \pm 2.0 \times 10^{-9}$ M and $1.25 \times 10^{-8} \pm 1.9 \times 10^{-9}$ M, respectively.

Part C

A significant increase in the development of active tension by rings of femoral artery from DOCA/NaCl hypertensive rats in a normal Krebs solution at 27° C in response to low concentrations of KCl (8 to 24 mM) was observed (Figure 53). Expressing the data as a percent of the maximal KCl response (Figure 54) reveals that the increase in responsiveness of femoral smooth muscle from DOCA/NaCl treated rats to KCl (8 to 32 mM) remains when compared to controls. The responses were similar to those at 37° C.

Maximal ISO-induced relaxation of rings of femoral artery from DOCA/NaCl hypertensive rats in normal Krebs at 27° C was significantly reduced (Figure 55). However, the sensitivity of femoral smooth muscle from DOCA/NaCl treated rats was significantly increased. The initial response to ISO occurred at 10^{-9} M for both groups with controls relaxing 2 percent and DOCA/NaCl rats relaxing 8 percent. Maximal relaxation occurred at 10^{-6} M ISO in DOCA/NaCl treated rats (31 percent relaxation) and at 10^{-5} M ISO in controls (49 percent relaxation). A 10 percent increase in tension was observed at 10^{-4} M ISO

Figure 52. Dose-response relationship between concentration of norepinephrine and percent of maximal norepinephrine response of rings of femoral artery from control and DOCA/NaCl hypertensive rats in high potassium (10 mM) Krebs physiological solution. Each response is calculated from the data in Figure 51 and is shown as a percent of the maximum active tension generated. No significant difference in the response of vascular smooth muscle to norepinephrine was observed between the two groups. Each point represents the mean and standard error of 12 rings.

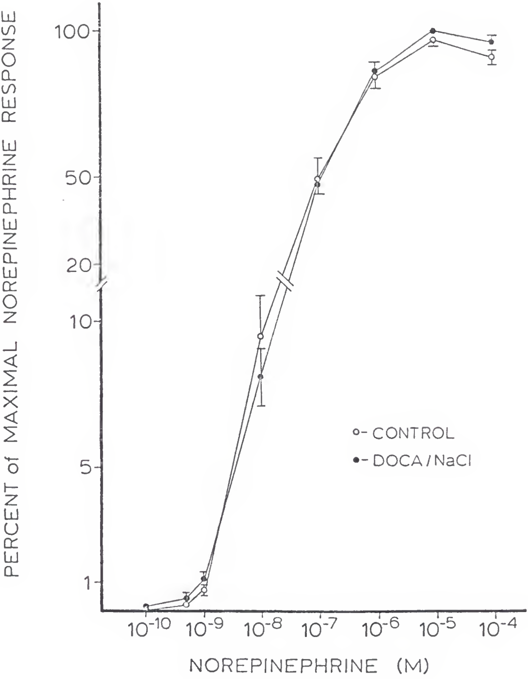

PERCENT of MAXIMAL NOREPINEPHRINE RESPONSE
100
50
20
10
5
1
10^-10
10^-9
10^-8
10^-7
10^-6
10^-5
10^-4
NOREPINEPHRINE (M)
○- CONTROL
●- DOCA / NaCl

Figure 53. Dose-response relationship between concentration of KCl and development of active tension by rings of femoral artery from control and DOCA/NaCl hypertensive rats at 27° C. A significant increase ($p < .05$) in the KCl sensitivity (8 to 24 mM) of vascular smooth muscle from DOCA/NaCl hypertensive rats was observed. Each point represents the mean and standard error of 12 rings.

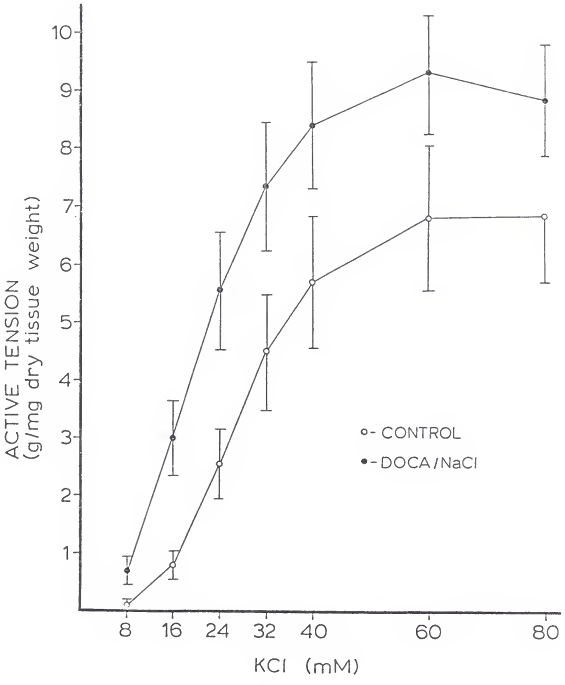

ACTIVE TENSION
(g/mg dry tissue weight)
10
9
8
7
6
5
4
3
2
1
8
16
24
32
40
60
80
KCl (mM)
○- CONTROL
●- DOCA/NaCl

Figure 54. Dose-response relationship between concentration of KCl and percent of maximal KCl response of rings of femoral artery from control and DOCA/NaCl hypertensive rats at 27° C. Each response is calculated from the data in Figure 53 and is shown as a percent of the maximum active tension generated. A significant increase ($p < .05$) in the response of vascular smooth muscle to KCl (8 to 32 mM) was observed in DOCA/NaCl hypertensive rats. Each point represents the mean and standard error of 12 rings.

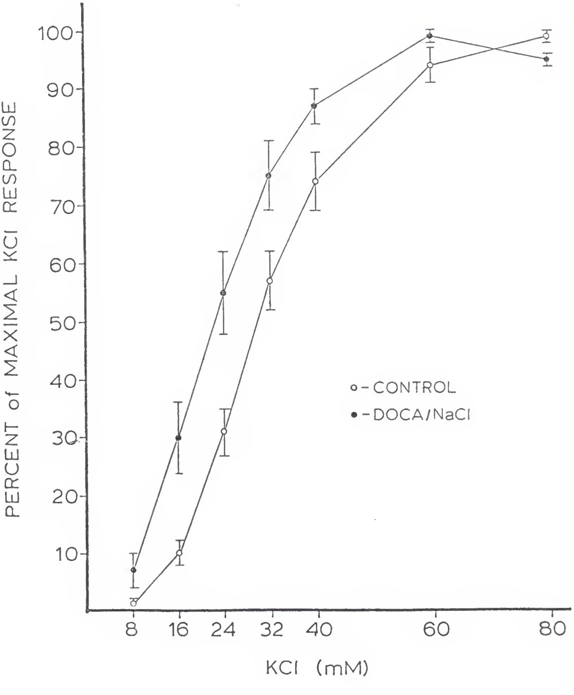
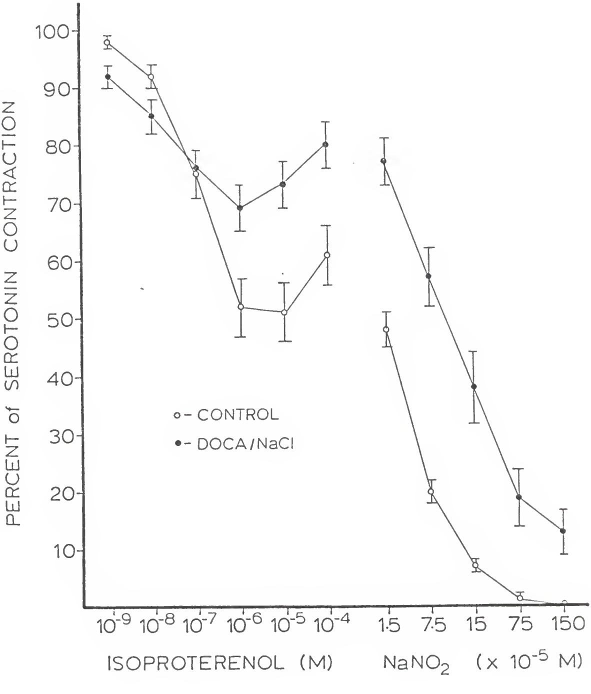

100
90
80
70
60
50
40
30
20
10
PERCENT of MAXIMAL KCl RESPONSE
8
16
24
32
40
60
80
KCl (mM)
○ - CONTROL
● - DOCA/NaCl

Figure 55. Dose-response relationship between concentration of isoproterenol and sodium nitrite and relaxation, expressed as a percent of serotonin contraction, of rings of femoral artery from control and DOCA/NaCl hypertensive rats at 27° C. Maximum isoproterenol-induced relaxation was significantly attenuated ($p < .01$) in vascular smooth muscle from DOCA/NaCl hypertensive rats. However, an increased sensitivity ($p < .05$) to isoproterenol (10^{-9} to 10^{-8} M) was observed in DOCA/NaCl hypertensive rats. A slight decrease in the maximal response to sodium nitrite was seen in DOCA/NaCl treated rats. Each point represents the mean and standard error of 12 rings.


PERCENT of SEROTONIN CONTRACTION
100
90
80
70
60
50
40
30
20
10
○ - CONTROL
● - DOCA/NaCl
10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5} 10^{-4}
1.5 7.5 15 75 150
ISOPROTERENOL (M)
$NaNO_2$ (x 10^{-5} M)

in control rats, while femoral smooth muscle from DOCA/NaCl rats showed an increase in tension at 10^{-5} M (4 percent increase) and 10^{-4} M ISO (11 percent increase). Maximal relaxation in response to $NaNO_2$ was significantly reduced in the DOCA/NaCl hypertensive rat with controls relaxing 100 percent and DOCA/NaCl relaxing only 87 percent.

A significant increase in both NE sensitivity and contractility occurred in rings of femoral artery from DOCA/NaCl hypertensive rats in normal Krebs solution at 27° C (Figure 56). Expressing the data as a percent of the maximal NE response (Figure 57) further shows the significant increase in NE sensitivity (10^{-10} to 10^{-7} M) as well as contractility (10^{-6} M). ED10 values for control and DOCA/NaCl hypertensive rats were $1.0 \times 10^{-8} \pm 1.9 \times 10^{-9}$ M and $5.0 \times 10^{-9} \pm 1.4 \times 10^{-9}$ M, respectively.

To more clearly show the effect of the sodium-potassium pump on NE responsiveness, the data at differing levels of pump activity within each group were graphed together. Figure 58 shows the response of VSM from controls in normal potassium (5.9 mM) Krebs, potassium-free Krebs, high potassium (10 mM) Krebs, and at 27° C. The NE response during complete inhibition of sodium-potassium pump activity (0 mM potassium) was increased at all concentrations of NE. The response in high potassium was slightly increased at high concentrations of NE (10^{-6} to 10^{-4} M) while no difference was observed at 27° C. Since there were differences in the maximal contractility

Figure 56. Dose-response relationship between concentration of norepinephrine and development of active tension by rings of femoral artery from control and DOCA/NaCl hypertensive rats at 27° C. A significant increase ($p < .05$ to $p < .001$) in the response of vascular smooth muscle from DOCA/NaCl hypertensive rats to norepinephrine (10^{-10} to 10^{-6} M) was observed. Each point represents the mean and standard error of 12 rings.

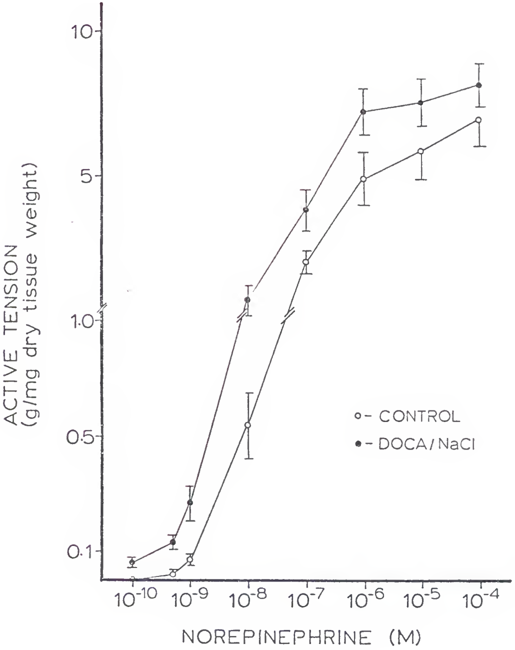

ACTIVE TENSION
(g/mg dry tissue weight)
10
5
1.0
0.5
0.1
10^-10
10^-9
10^-8
10^-7
10^-6
10^-5
10^-4
NOREPINEPHRINE (M)
○ - CONTROL
● - DOCA / NaCl

Figure 57. Dose-response relationship between concentration of norepinephrine and percent of maximal norepinephrine response of rings of femoral artery from control and DOCA/NaCl hypertensive rats at 27° C. Each point is calculated from the data in Figure 56 and is shown as a percent of the maximum active tension generated. A significant increase ($p < .05$ to $p < .001$) in the response of vascular smooth muscle from DOCA/NaCl hypertensive rats to norepinephrine (10^{-10} to 10^{-6} M) was observed. Each point represents the mean and standard error of 12 rings.

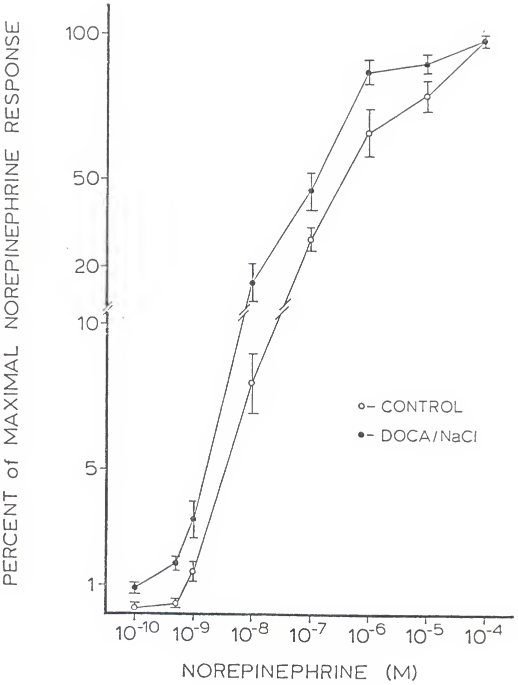
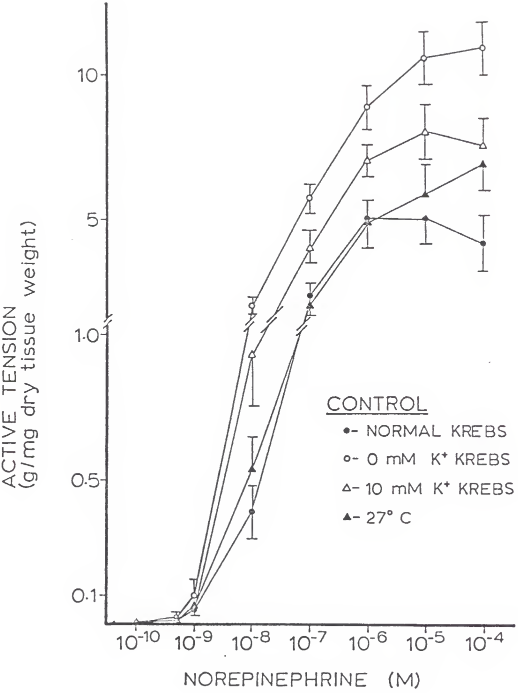

PERCENT of MAXIMAL NOREPINEPHRINE RESPONSE
100
50
20
10
5
1
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
NOREPINEPHRINE (M)
○– CONTROL
●– DOCA/NaCl

Figure 58. Dose-response relationship between concentration of norepinephrine and development of active tension by rings of femoral artery from control rats at 27° C or in either a potassium-free (0 mM), high potassium (10 mM), or normal potassium (5.9 mM) Krebs physiological solution. A significant increase ($p < .05$) in the response of vascular smooth muscle to norepinephrine (10^{-8} to 10^{-4} M) in the potassium-free Krebs solution was observed. Each point represents the mean and standard error of 12 rings.


ACTIVE TENSION
(g/mg dry tissue weight)
10
5
1.0
0.5
0.1
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
NOREPINEPHRINE (M)
CONTROL
●- NORMAL KREBS
○- 0 mM K+ KREBS
△- 10 mM K+ KREBS
▲- 27° C

at each level of pump activity, the responses were normalized and expressed as a percent of the maximal NE response (Figure 59). At low concentrations of NE (10^{-10} to 10^{-8} M), VSM exhibited an increased sensitivity in the potassium-free Krebs. At 27° C the sensitivity to NE was also slightly increased, but not at much as in the potassium-free Krebs. No difference was observed in the high potassium Krebs.

The alterations in reactivity of VSM from DOCA/NaCl hypertensive rats to NE were more pronounced than in controls in response to variations in the activity of the sodium-potassium pump. Figures 60 and 61 show that the response to NE is dependent upon the activity of the sodium-potassium pump. Decreasing the activity of the pump by approximately 50 percent (27° C) results in an increase in the sensitivity of the VSM to NE. Maximal pump inhibition (potassium-free Krebs) causes a further increase in the response to NE, while maximal pump stimulation (10 mM potassium Krebs) results in a decrease in the sensitivity to NE. The effect of altering sodium-potassium pump activity on α-adrenergic responsiveness is further evident in the ED10 values (Table 4).

Figure 62 shows the ISO dose-response curves for VSM from controls in 0 mM potassium (potassium-free) Krebs, normal potassium (5.9 mM) Krebs, high potassium (10 mM) Krebs, and at 27° C. The response to ISO was similar for all groups except for a slight decrease in the maximal

Figure 59. Dose-response relationship between concentration of norepinephrine and percent of maximal norepinephrine response of rings of femoral artery from control rats at 27° C or in either a potassium-free (0 mM), a high potassium (10 mM), or normal potassium (5.9 mM) Krebs physiological solution. Each response is calculated from the data in Figure 58 and is shown as a percent of the maximum active tension generated. A significant increase ($p < .05$) in the sensitivity of vascular smooth muscle to norepinephrine (5×10^{-10} to 10^{-8} M) was observed in potassium-free Krebs solution. Each point represents the mean and standard error of 12 rings.

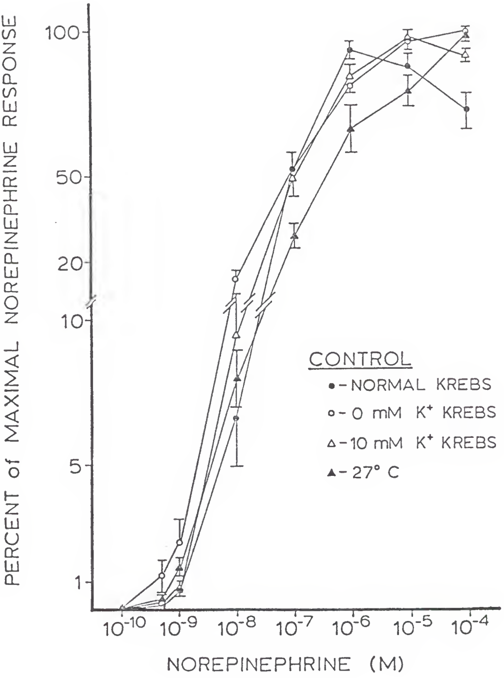

PERCENT of MAXIMAL NOREPINEPHRINE RESPONSE
100
50
20
10
5
1
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
NOREPINEPHRINE (M)
CONTROL
● - NORMAL KREBS
○ - 0 mM K^+ KREBS
△ - 10 mM K^+ KREBS
▲ - 27° C

Figure 60. Dose-response relationship between concentration of norepinephrine and development of active tension by rings of femoral artery from DOCA/NaCl hypertensive rats at 27° C or in either a potassium-free (0 mM), high potassium (10 mM), or a normal potassium (5.9 mM) Krebs physiological solution. Responses of vascular smooth muscle to norepinephrine (10^{-10} to 10^{-9} M) in potassium-free Krebs or at 27° C were significantly greater ($p < .05$) than responses in normal or high potassium Krebs. Maximal contractility to norepinephrine (10^{-6} to 10^{-4} M) was significantly greater ($p < .05$) in the high potassium Krebs. Each point represents the mean and standard error of 12 rings.

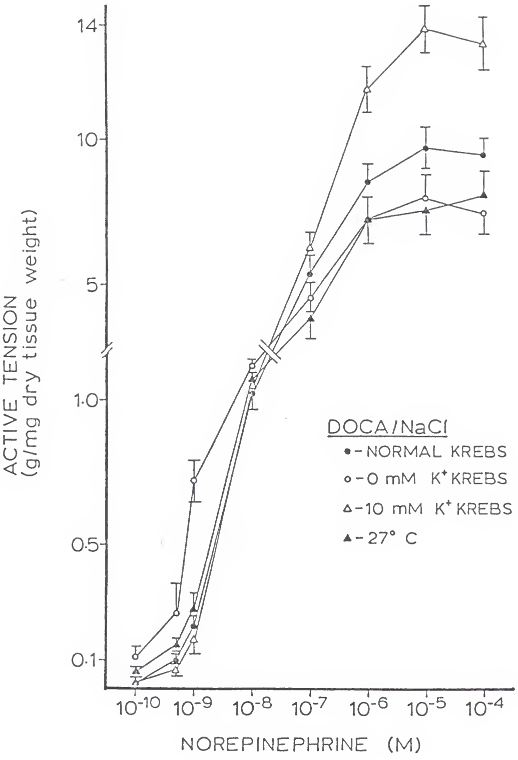

ACTIVE TENSION
(g/mg dry tissue weight)
14
10
5
1.0
0.5
0.1
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
NOREPINEPHRINE (M)
DOCA/NaCl
● - NORMAL KREBS
○ - 0 mM K⁺ KREBS
△ - 10 mM K⁺ KREBS
▲ - 27° C

Figure 61. Dose-response relationship between concentration of norepinephrine and percent of maximal norepinephrine response of rings of femoral artery from DOCA/NaCl hypertensive rats at 27° C or in either a potassium-free (0 mM), high potassium (10 mM), or normal potassium (5.9 m) Krebs physiological solution. Each response is calculated from the data in Figure 60 and is shown as a percent of the maximum active tension generated. A significant increase ($p < .01$) in the sensitivity of vascular smooth muscle to norepinephrine (10^{-10} to 10^{-9} M) was observed in potassium-free Krebs solution when compared to all other groups. The response to norepinephrine (10^{-10} to 10^{-9} M) at 27° C was less than in the potassium-free Krebs solution but significantly greater ($p < .05$) than responses in normal or high potassium Krebs. The sensitivity to norepinephrine (5×10^{-10} to 10^{-8} M) in the high potassium Krebs was significantly decreased ($p < .05$) when compared to all other groups. Each point represents the mean and standard error of 12 rings.

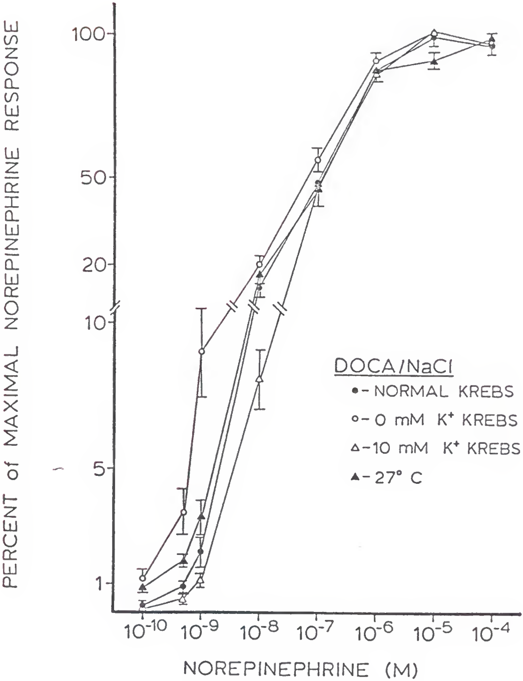

PERCENT of MAXIMAL NOREPINEPHRINE RESPONSE
100
50
20
10
5
1
10-10
10-9
10-8
10-7
10-6
10-5
10-4
NOREPINEPHRINE (M)
DOCA/NaCl
●- NORMAL KREBS
○- 0 mM K+ KREBS
△-10 mM K+ KREBS
▲- 27° C

TABLE 4. EFFECTIVE DOSE OF NOREPINEPHRINE WHICH RESULTS IN A 10 PERCENT OF MAXIMAL RESPONSE (ED10) IN RINGS OF FEMORAL ARTERY FROM CONTROL AND DOCA/NaCl HYPERTENSIVE RATS AT VARIOUS LEVELS OF EXTRACELLULAR POTASSIUM CONCENTRATION AND AT 27° C

Treatment Group	Extracellular Potassium Concentration			
	0 mM	5.9 mM	10 mM	27° C
Control	4.9×10^{-9} $\pm 1.0 \times 10^{-9}$ M[a,c,d]	1.3×10^{-8} $\pm 2.1 \times 10^{-9}$ M	1.05×10^{-8} $\pm 2.0 \times 10^{-9}$ M	1.0×10^{-8} $\pm 1.9 \times 10^{-9}$ M
DOCA/ NaCl	1.25×10^{-9} $\pm 0.9 \times 10^{-9}$ M[b,c]	6.4×10^{-9} $\pm 1.5 \times 10^{-9}$ M[b,d]	1.25×10^{-8} $\pm 1.9 \times 10^{-9}$ M	5.0×10^{-9} $\pm 1.4 \times 10^{-9}$ M[b,d]

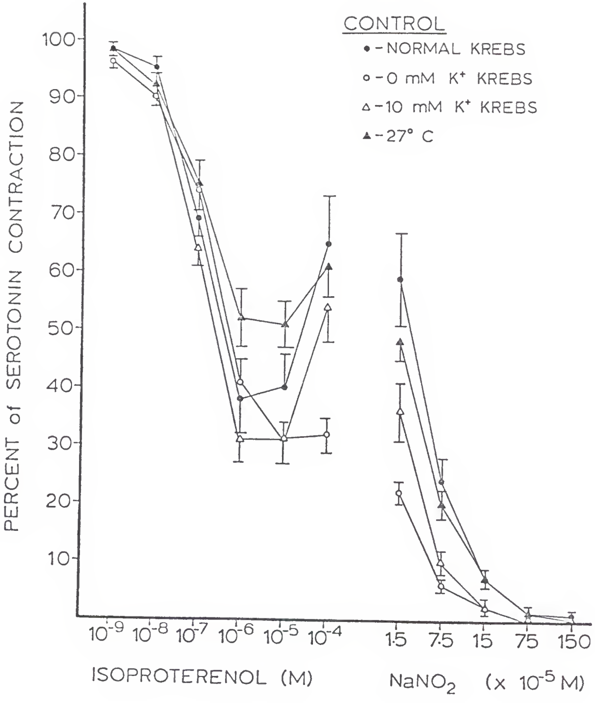
[a] Mean ± SEM

[b] Significantly different from control ($p < .05$)

[c] Significantly different from values at 5.9 mM, 10 mM, and 27° C within the respective treatment group ($p < .05$)

[d] Significantly different from values at 10 mM within the respective treatment group ($p < .05$)

Figure 62. Dose-response relationship between concentration of isoproterenol and sodium nitrite and relaxation, expressed as a percent of serotonin contraction, of rings of femoral artery from control rats at 27° C or in either a potassium-free (0 mM), high potassium (10 mM), or normal potassium (5.9 mM) Krebs physiological solution. Maximal isoproterenol-induced relaxation of vascular smooth muscle was significantly attenuated ($p < .05$) at 27° C when compared to all groups. No difference in the maximum response to sodium nitrite was observed. Each point represents the mean and standard error of 12 rings.


CONTROL
● - NORMAL KREBS
○ - 0 mM K+ KREBS
△ - 10 mM K+ KREBS
▲ - 27° C
PERCENT of SEROTONIN CONTRACTION
100
90
80
70
60
50
40
30
20
10
10-9 10-8 10-7 10-6 10-5 10-4
1.5 7.5 15 75 150
ISOPROTERENOL (M)
NaNO2 (x 10-5 M)

response at 27° C. Relaxation in response to $NaNO_2$ was similar for all groups.

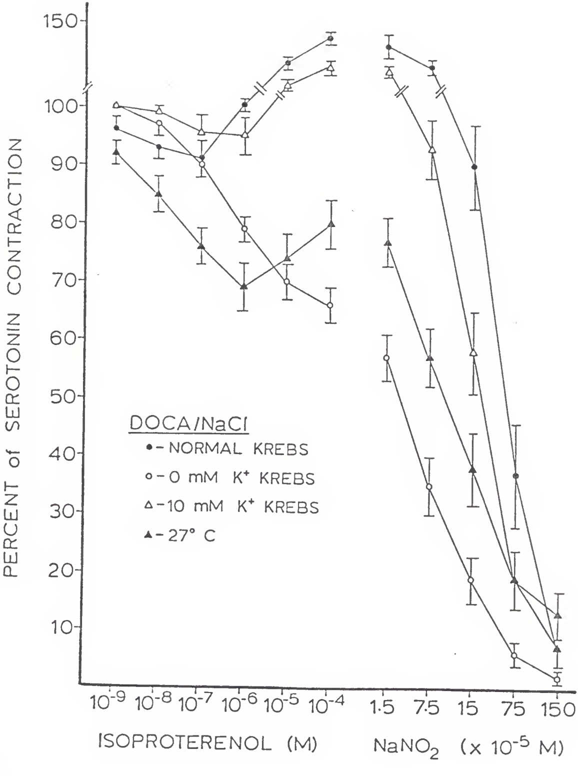
The response of VSM from DOCA/NaCl treated rats to ISO at various levels of sodium-potassium pump activity was quite complex (Figure 63). Maximal relaxation (34 percent) in response to ISO was greater in VSM incubated in the potassium-free Krebs solution (maximal pump inhibition), and was least (4 percent relaxation) in the 10 mM potassium Krebs (maximal pump stimulation). Vascular smooth muscle incubated at 27° C (50 percent inhibition of pump activity) responded similar to that in 0 mM potassium (30 percent relaxation), while the response in the normal Krebs (8 percent relaxation) was slightly greater than that in the high potassium Krebs. Maximal relaxation in response to $NaNO_2$ was similar for all groups.

Discussion

The experiments in this section were designed to determine the role of the sodium-potassium pump in mediating adrenergic responsiveness in VSM and if the alterations in vascular reactivity of the DOCA/NaCl hypertensive rat are associated with an alteration in the activity of the sodium-potassium pump.

As discussed in the Introduction to this section, studies have shown that NE responsiveness in VSM is dependent, to a degree, on the membrane potential (Mulvany *et al.*, 1982a). In addition, the level of activity of the

Figure 63. Dose-response relationship between concentration of isoproterenol and sodium nitrite and relaxation, expressed as a percent of serotonin contraction, of rings of femoral artery from DOCA/NaCl hypertensive rats at 27° C or in either a potassium-free (0 mM), high potassium (10 mM), or normal potassium (5.9 mM) Krebs physiological solution. Maximal isoproterenol-induced relaxation of vascular smooth muscle was significantly greater ($p < .01$) in the potassium-free Krebs solution and at 27° C when compared to responses in high or normal potassium. No difference in the maximum response to sodium nitrite was observed. Each point represents the mean and standard error of 12 rings.


PERCENT of SEROTONIN CONTRACTION
150
100
90
80
70
60
50
40
30
20
10
DOCA/NaCl
●- NORMAL KREBS
○- 0 mM K⁺ KREBS
△-10 mM K⁺ KREBS
▲- 27° C
10⁻⁹ 10⁻⁸ 10⁻⁷ 10⁻⁶ 10⁻⁵ 10⁻⁴
ISOPROTERENOL (M)
1·5 7·5 15 75 150
NaNO₂ (x 10⁻⁵ M)

sodium-potassium pump appears to have an effect on VSM responsiveness to NE (Skaug and Detar, 1981a, 1981b).

A few studies have been done in the SHR concerning the sodium-potassium pump and its role in altered vascular reactivity (Mulvany et al., 1980b; Gothberg et al., 1980; Nilsson and Mulvany, 1981). From these studies it has been suggested that the increased VSM responsiveness to NE in SHR is due, in part, to the altered activity of the sodium-potassium pump.

As opposed to the SHR, however, the role of the sodium-potassium pump in altering vascular adrenergic responsiveness in the DOCA/NaCl hypertensive rat has not been reported elsewhere. The results of this study suggest an important role for the sodium-potassium pump in mediating the increased α-adrenergic responsiveness of VSM in DOCA/NaCl rats. Its role in mediating vascular β-adrenergic responsiveness, however, appears to be less well defined.

Besides altering the membrane potential and thus, increasing the excitability of the VSM cell to NE stimulation (discussed in the Introduction of this section), alterations in sodium-potassium pump activity may increase the sensitivity of VSM through other mechanisms. These mechanisms were described in detail in the Introduction of Part II, however, they will be briefly reviewed again.

The sodium-potassium pump may affect VSM tone and increase vascular reactivity through a sodium-calcium exchange. Inhibition of the pump results in an increase in intracellular sodium. This sodium then exchanges with extracellular calcium which increases the level of available intracellular calcium for VSM contraction. Upon stimulation with NE this results in an enhanced response.

The results of Experiment III showed that the increased sensitivity to NE in the DOCA/NaCl treated rat could be diminished in a low calcium Krebs. It was suggested that an increased permeability of the VSM cell to this cation resulted in the enhanced responsiveness. It may be possible that the increased response to NE (and increased "permeability" to calcium) is due, in part, to an alteration in the sodium-potassium pump that contributes to the increased calcium sensitivity of the VSM. Mulvany _et al_. (1980) have shown in the SHR that the increase in NE-activated calcium sensitivity of VSM is associated with alterations in the sodium-potassium pump. Similar responses may occur in the DOCA/NaCl hypertensive rat as well.

Another mechanism by which an alteration in sodium-potassium pump activity can increase VSM is through the release of endogenous NE. Inhibition of the pump in adrenergic nerve terminals causes a depolarization and release of NE as well as prevents the active uptake of NE into the terminal. This would result in an increased response to exogenous NE also.

Evidence has been put forth for the role of adrenergic nerve terminals in the increase in response of VSM to NE in vitro. In order to abolish any role for the nerve terminal in the response to exogenous NE, most investigators either destroy the adrenergic nerve terminals (Webb et al., 1981b) or use cocaine to block the uptake of NE into the terminal (Webb et al., 1983b). In the DOCA/NaCl hypertensive rat it is possible that the increased response to NE is due to a release of endogenous NE as a result of an alteration in the sodium-potassium pump in the adrenergic nerve terminal.

However, since it was found in preliminary studies that responses to NE were not appreciably affected by the presence of adrenergic nerve terminals, the terminals were not destroyed in the present study. Therefore, it appears that the adrenergic nerve terminals do not play a significant role in the increased NE responsiveness of VSM from the DOCA/NaCl rat. In addition, the role of the sodium-potassium pump in altering VSM reactivity to NE must occur through one of the other mechanisms mentioned above, most likely the sodium-calcium exchange.

One problem arises, however, in explaining the role of an altered sodium-potassium pump in the increase in VSM responsiveness to NE. It was reported in the second part of this dissertation that sodium-potassium pump activity was increased in VSM from DOCA/NaCl hypertensive rats. Since it was shown in this experiment that NE sensitivity was decreased when pump activity was increased (10 mM potassium Krebs), then the response of VSM from the DOCA/NaCl treated rat to NE should actually be decreased and not increased. Therefore, based upon the above arguments, it appears that the increase in VSM NE sensitivity observed in the DOCA/NaCl rat is more likely to be due to some other factor, such as an increase in VSM permeability to calcium, as discussed in Part I. However, a role for the sodium-potassium pump should not be totally ruled out.

Pamnani _et_ _al_. (1981b) and Kojima _et_ _al_. (1982) have demonstrated the existence of a humoral, digitalis-like substance in DOCA/NaCl hypertensive rats which inhibits the VSM sodium-potassium pump. In addition, Pamnani _et_ _al_. (1980a) have shown a decrease in pump activity of VSM from the DOCA/NaCl hypertensive rats, as opposed to an increase reported here. It has been suggested that the differences in results obtained are due to differences in the experimental methods. That is, Pamnani's group measure sodium-potassium pump activity using ^{86}Rb uptake shortly after removal of the VSM from the animal. Others, however, incubate the tissue for a couple of hours prior to the study. It has been suggested that the longer incubation period allows enough time for the endogenous digitalis-like factor to be washed out, and therefore, an increase, as opposed to a decrease, in pump activity occurs. The increase in pump activity, when compared to controls, may be due to a compensatory mechanism on the part of the VSM to increase the number of pump sites in an attempt to offset the effects of the endogenous sodium-potassium pump inhibitor. This is similar to an upregulation of receptors and would be done in an attempt to maintain normal ion movements, and thus membrane potential, in the VSM cell.

If there is a circulating inhibitor of the VSM sodium-potassium pump *in vivo*, then an increase in the response to NE as a result of pump inhibition is a definite possibility. It is suggested, therefore, that an alteration in the sodium-potassium pump does play an important role in contributing to the increase in peripheral vascular resistance observed in the DOCA/NaCl treated rat by increasing the sensitivity of the VSM to NE stimulation.

Although the time course of alterations in sodium-potassium pump activity and the occurrence of this digitalis-like factor are not known, it may well be that they are involved in the early developmental stages of DOCA/NaCl hypertension and contribute to the initial increase in vascular resistance through an increase in NE sensitivity.

Based upon the studies by Webb and Bohr (1981) and Broekaert and Godfraind (1973) using normotensive animals, inhibition of the sodium-potassium pump results in a decrease in ISO-induced relaxation. The results of this study, however, agree with that of Mueller and van Breemen (1979) in that the pump appears not to be involved in the relaxation induced by ISO. No significant effect of altered potassium or decrease in temperature was observed on ISO-induced relaxation of VSM from controls. The response in VSM from DOCA/NaCl hypertensive rats, however, is puzzling.

The increase in response to ISO in potassium-free Krebs as well as the decrease in response in 10 mM potassium are paradoxical to the findings of Webb and Bohr (1981). It would be expected that if the sodium-potassium pump plays even a minor role in β-adrenergic mediated relaxation, the response would be slightly decreased in potassium-free Krebs when compared to normal potassium. This, of course, was not the case.

Since the ISO dose-response curves were carried out following a contraction with serotonin, it may be reasonable to suggest that there is some complex interaction occurring between ISO, serotonin and the level of pump activity.

The actions of serotinin itself appear to be quite complex. It is known to act at several different types of receptors initiating various responses in the same tissue (Vanhoutte, 1983). One of these receptor types is the β-adrenergic receptor. However, its interaction with this receptor appears to be minimal or even nonexistent in some VSM.

Serotonin is also known to produce tachyphylaxis in VSM (Collis and Vanhoutte, 1981). Although the mechanism behind the tachyphylaxis is not known, it may be possible that the activity of the sodium-potassium pump is involved.

The tachyphylactic response to serotonin is delayed in the SHR. In addition, it is known that sodium-potassium pump activity is increased in VSM from SHR. Therefore, by decreasing pump activity, the tachyphylaxis may be increased. In the present study, VSM from the DOCA/NaCl hypertensive rat in 0 mM potassium exhibited a progressive decrease in tension. This decrease in tension could actually be due to a tachyphylactic response of serotonin and not a true ISO-induced relaxation.

Whatever the mechanism behind this complex response of VSM from DOCA/NaCl hypertensive rats, it appears that the sodium-potassium pump does not play a significant role in the decrease in vascular β-adrenergic responsiveness observed in this form of hypertension. In summary, these studies have shown that alterations in sodium-potassium pump activity can affect VSM responsiveness to NE but not ISO. In addition, and more importantly, it was shown that alterations in the sodium-potassium pump have a much greater effect on α-adrenergic responsiveness of VSM from the DOCA/NaCl rat than controls. Finally, it is suggested that the alteration in pump activity in the DOCA/NaCl hypertensive rat may be an important contributory factor to the increase in peripheral vascular resistance by increasing the sensitivity of the VSM to NE.

PART VI
SERUM THYROID HORMONE CONCENTRATION IN DOCA/NaCl HYPERTENSIVE RATS

Introduction

The development and maintenance of various forms of experimental hypertension appears to be influenced by a number of humoral and neurogenic factors. The thyroid hormones have been implicated as one of these factors.

Fregly (1959) observed that rats fed a hypertonic saline solution (1.75 percent) developed an increase in systolic blood pressure significantly higher than controls. By administering the antithyroid drug propylthiouracil (PTU) concomittantly with the high salt diet the increase in blood pressure was prevented.

Salgado and Selye (1952) reported an inhibition of the development of hypertension induced by desoxycorticosterone acetate (DOCA) and a high sodium intake in hypophysectomized rats. To further determine which hypophyseal factor may be involved, Salgado (1954) studied the effects of thyroidectomy on the development and maintenance of DOCA/salt hypertension. It was observed that animals thyroidectomized at the onset of DOCA administration did not develop an increase in blood pressure, while thyroidectomy performed after the development of hypertension resulted in a decrease in blood pressure.

Renal hypertension in the rat induced by bilateral encapsulation of the kidneys was prevented by the administration of PTU (Fregly, 1958; Fregly and Cook, 1960). Addition of dessicated thyroid to the diet overcame the antihypertensive effects of PTU and resulted in an increase in blood pressure. Administration of PTU or methimazole after the hypertensive state was achieved resulted in a decrease in blood pressure to normotensive levels. Thyroidectomy had similar effects (Fregly et al., 1960). Fregly and Gonzalez (1961) have suggested that the thyroid gland plays a secondary role in the development of renal hypertension in the rat. The ratio of thyroid weight to body weight remains constant until blood pressure reaches levels of 150-160 mm Hg pressure whereupon a linear increase occurs. It has also been shown that rats whose thyroid glands are removed just prior to encapsulation of the kidney showed rises in systolic blood pressure from initial levels of approximately 130 mm Hg to approximately 155 mm Hg. Therefore, thyroid involvement in the development of renal hypertension may not become important until the systolic blood pressure reaches levels of 150-160 mm Hg.

Considerable evidence has been accumulated indicating involvement of the thyroid gland in development of hypertension in the spontaneously hypertensive rat (SHR). It is well established that ablation of the thyroid or administration of PTU in the SHR before the onset of hypertension will prevent the increase in blood pressure (Rioux and Berkowitz, 1977; Vincent et al., 1978; Yamabe, 1970). Thyroidectomy, administration of PTU, or injection of radioactive iodine progressively reduce the high blood pressure of adult SHR's toward the normotensive level. Replacement doses of thyroxine (T_4) causes a recurrence of hypertension in these animals (Rioux and Berkowitz, 1977). Yamabe (1970) and Kojima et al. (1976) have shown an increase in thyroid activity of the SHR as measured by ^{131}I uptake and release, whereas Fregly (1975) has observed a decrease. When compared to controls, both Yamabe (1970) and Fregly (1975) observed a greater T_4 secretion rate suggesting an increase in TSH release. Kojima et al. (1976) saw a decrease in plasma T_4 levels even though TSH secretion was increased. Rioux and Berkowitz (1977) have observed low plasma concentrations of T_4 in SH rats in the prehypertensive period which increase to normal levels during the hypertensive process.

A number of biological compounds have been proposed as regulators of Na,K-ATPase either through their effects on the biosynthesis of the enzyme or on the enzyme's activity. A well investigated example of this is the thyroid hormones which have been shown to affect the synthesis of this enzyme. Ismail-Beigi and Edelman (1970) have carried out a number of studies correlating thyroid calorigenesis and active sodium transport. Using liver slices, kidney slices, and skeletal muscle (Ismail-Beigi and Edelman, 1971) they were able to show that a large percentage of the increase in oxygen consumption produced by injection of thyroid hormone into euthyroid and hypothyroid rats was from an increase in sodium pump activity. Na,K-ATPase activity, determined by the amount of inorganic phosphate libertated, showed a significant increase with triiodothyronine (T_3) administration. A decrease in intracellular sodium and an increase in intracellular potassium was observed in diaphragm and heart tissue of euthyroid and thyroidectomized rats injected with T_3 (Ismail-Beigi and Edelman, 1973). This is consistent with the enhancement of active sodium transport by thyroid hormone. Evidence for an increase in the synthesis of Na,K-ATPase was shown by the study of Lo and Edelman (1976) in which there was a T_3-stimulated increase in the incorporation of radiolabeled amino acids into the enzyme. T_3 administration to guinea pigs resulted in an increase in myocardial Na,K-ATPase activity, an increase in ^{86}Rb uptake by atrial strips, and an increase in ouabain binding by atrial and ventricular homogenates (Curfman, et al., 1977). Philipson and Edelman (1977a, 1977b) have shown a time dependent increase in rat myocardial Na,K-ATPase activity after T_3 treatment with significant effects occurring at 24-48 hours. Intracellular potassium levels also increased in correspondence with this time course. Veroni and Shenfield (1980) showed that the accumulation of ouabain in hearts of hyperthyroid rats was greater than that of euthyroid rats and suggests an increase in the number of Na,K-ATPase sites.

Since the thyroid gland appears to play a role in the development of many forms of experimental hypertension, and the thyroid hormones have an effect on the number of sodium-potassium pump sites in various tissues, serum thyroid hormone levels will be determined in the DOCA/NaCl hypertensive rat. This will be done to assess whether a possible alteration in the thyroid state of the animal may have an effect on the vascular sodium-potassium pump and thus affect vascular reactivity.

Experiment VIII

This experiment examined the serum concentrations of triiodothyronine (T_3) and thyroxine (T_4) of control and DOCA/NaCl hypertensive rats.

Methods

Serum samples were taken from control and DOCA/NaCl hypertensive rats at the time of sacrifice as described in General Methods. Serum was assayed for total T_4 and T_3 using RIA Coat-a-Count kits from Diagnostic Products Corp. (Los Angeles, CA).

Results

A significant decrease ($p < .01$) in the concentration of serum T_4 was seen in the DOCA/NaCl hypertensive rat when compared to controls (2.75 ± 0.28 μl/dl vs. 4.22 ± 0.45 μg/dl). Although serum T_3 concentration was slightly increased in the DOCA/NaCl hypertensive rat, it was not significant (75 ± 6 ng/dl vs. 58 ± 7 ng/dl).

Discussion

The results of this assay on thyroid hormone concentrations suggest an alteration in the thyroid state of the DOCA/NaCl hypertensive rat.

The reduction of T_4 could be the result of any one of several alterations in the regulation of thyroid hormone levels. One possible mechanism could involve the hypothalamo-pituitary-thyroid axis. First, a defect in T_4 synthesis by the thyroid gland could be implicated, resulting in a decrease in the secretion of T_4. Secondly, the thyroid gland itself may not be altered in its ability to produce and secrete T_4, but there may be a decrease in the stimulation of the thyroid gland by the pituitary hormone, thyroid stimulating hormone (TSH). A decrease in the release of TSH by the pituitary would lead to subsequent decrease in T_4 secretion. Normally the release of TSH is regulated by the levels of thyroid hormones. A decrease in serum concentration of T_3 and/or T_4 has an effect on the pituitary to increase TSH release. This increase results in a stimulation of the thyroid to synthesize and secrete T_3 and T_4. However, if a defect in the pituitary results in an inability of the decrease in thyroid hormone to stimulate TSH release, then T_3 and T_4 will remain low.

The defect may not be in the pituitary, however. An alteration in the hypothalamus, which releases the hormone, thyrotropin releasing hormone (TRH) and controls the release of TSH from the pituitary, may occur also. Again, a decrease in TRH would also lead to a decrease in T_4 secretion.

A second mechanism by which a decrease in serum T_4 may occur is through an alteration in the metabolism of the hormone. That is, the conversion of T_4 to other thyroid hormones, such as T_3, may be increased in the DOCA/NaCl rat. It is suggested that the decrease in T_4 is due to an increase in the conversion to T_3 since slight elevations in T_3 occurred. Based upon these results, it appears unlikely that an alteration in the hypothalamo-pituitary-thyroid axis exists.

One reason for this suggestion comes from the data on thyroid weight. Thyroid glands from DOCA/NaCl hypertensive rats were significantly greater than controls (Table 1). This is most likely due to the decrease in serum thyroid hormone (T_4) which results in an increase in the release of TSH. Thyroid stimulating hormone has several effects upon the thyroid, one of which is to increase the number and size of secretory cells in the thyroid gland.

Another reason for this suggestion is that if a defect occurred somewhere in the hypothalamo-pituitary-thyroid axis, it would be expected that both T_4 and T_3 would be affected in a similar fashion. Therefore, it appears that the feedback system to regulate thyroid hormone secretion is intact.

Unfortunately, there have been no studies reported, to the knowledge of this author, on thyroid activity in the DOCA/NaCl hypertensive rat. Several studies have been done on the SHR measuring thyroid hormones, TSH and thyroid activity. The results from these studies are conflicting, however. A few of these studies were mentioned in the Introduction to this section. Some investigators have reported increased secretion of T_4 (Yamabe, 1972; Fregly, 1975), while others have observed decreased T_4 even though TSH was increased. In the SHR, this suggests a defect in the pituitary-thyroid axis.

Whatever the mechanism, it is apparent that an alteration in the thyroid state of the DOCA/NaCl hypertensive rat exists.

It is emphasized by this author, though, that the present study was merely designed to determine serum T_3 and T_4 concentrations of the DOCA/NaCl hypertensive rat and to possibly correlate any alterations in thyroid hormone levels with the VSM sodium-potassium pump. It was suggested that the activity and/or number of sodium-potassium pumps in VSM may be affected by the thyroid hormones. It has been established that both T_4 and T_3 can affect sodium-potassium activity in various tissues. If this were the case, it could be expected that the activity of the sodium-potassium pump in DOCA/NaCl hypertensive rats would be decreased since the overall level of T_4 and T_3 are relatively decreased. However, no studies have been done investigating the effect of T_3 or T_4 on VSM sodium-potassium pump activity.

Although the thyroid gland is a requirement for the development of hypertension in the DOCA/NaCl treated rat, the mechanisms or contributions involved in the increase in peripheral resistance are not known.

From this study it can be concluded that alterations in thyroid hormone occur in the DOCA/NaCl hypertensive rat, but their possible contribution to the development of hypertension is not known. It can only be postulated at this time that the thyroid hormones may affect sodium-potassium pump activity thereby altering the responsiveness of the VSM and increasing peripheral vascular resistance.

PART VII
CONCLUSION

The following conclusions represent interpretations by this author of the experiments performed in this dissertation.

The first part of this dissertation was concerned with alterations in vascular reactivity of femoral smooth muscle in the DOCA/NaCl hypertensive rat and the role of extracellular calcium in these changes.

Measurement of sodium-potassium pump activity was performed in the second part of this dissertation in femoral arteries from control S-D rats and DOCA/NaCl hypertensive rats.

In the third part of this dissertation, experiments were performed to investigate the role of the sodium-potassium pump in the alterations of vascular reactivity in DOCA/NaCl hypertensive rats.

Finally, serum T_3 and T_4 were measured in control and DOCA/NaCl rats at the time of experimentation to determine the possibility of a role for altered thyroid hormone in the changes in sodium-potassium pump activity.

In the initial studies on vascular reactivity it was observed that VSM from DOCA/NaCl hypertensive rats exhibited an enhanced reactivity to KCl and NE, while the

the response to ISO was decreased. The increased response to KCl suggests an increase in the calcium sensitivity or permeability of the VSM cell, since KCl causes a contraction which is dependent upon the influx of calcium. This is supported by the findings in Experiment III in which KCl dose-response curves were generated in a low calcium (0.25 mM) Krebs solution. Although VSM from control animals showed a decrease in the response to KCl, femoral rings from DOCA/NaCl treated rats were affected to a greater degree and actually responded much less than controls.

A further role for extracellular calcium in the increase in vascular reactivity in DOCA/NaCl hypertensive rats becomes apparent from the observations that low calcium decreased the response of rings of femoral arteries from DOCA/NaCl rats to NE. Since the response was identical to that of controls in low calcium it can be suggested that no alterations in intracellular calcium mobilization occurs in VSM from the DOCA/NaCl rat. If there was a defect in intracellular calcium stores, one would expect the response of VSM from DOCA/NaCl animals in low calcium to be somewhat different than controls, since NE contraction is dependent upon both intra- and extracellular calcium stores.

As opposed to KCl and NE, the decrease in ISO-induced, β-adrenergic mediated relaxation in DOCA/NaCl hypertensive rats does not appear to be the result of altered calcium handling in the VSM cell. This is suggested since low calcium did not appreciably affect ISO-induced relaxation and no difference in the response to $NaNO_2$, a nonspecific smooth muscle relaxant, was observed between controls and DOCA/NaCl hypertensive rats.

Therefore, from the first part of this dissertation it can be concluded that extracellular calcium plays an important role in the increase in vascular reactivity, and more importantly NE sensitivity of VSM, from the DOCA/NaCl hypertensive rat. This may be an important factor in the increase in peripheral vascular resistance and development of hypertension in the DOCA/NaCl treated rat since it has been observed that the increase in NE sensitivity occurs prior to the actual onset of hypertension.

Sodium-potassium pump activity in VSM from DOCA/NaCl hypertensive rats was significantly increased over controls. This finding is in agreement with some investigators but is in conflict with others. It has been suggested that the differences in observation are due to differences in experimental design. In this dissertation, tissues were preincubated for 60-90 minutes prior to actual measurement of pump activity. During this incubation, a postulated ouabain-like humoral factor may be washed out, thus allowing for the expression of an increased number and/or activity of pumps. The increased number and/or activity of sodium-potassium pumps may be the result of a couple of factors. One is an increase in the passive permeability of the VSM cell, while another is the postulated ouabain-like humoral factor. Both are suggested to increase the activity of the pump through a compensatory mechanism. Since no alteration in passive rubidium-86 uptake was observed, it is felt by this author that the increase in pump activity is a compensation by the VSM cell in response to the postulated ouabain-like factor. This has been suggested also by Overbeck and Grissette (1982).

The response of VSM from controls and DOCA/NaCl hypertensive rats to NE was increased in the presence of a potassium-free Krebs solution. The response, however, was greater in the DOCA/NaCl group. In addition, rings of femoral artery from DOCA/NaCl treated rats exhibited an increase in the response to NE at 27° C and a decrease in response in 10 mM potassium. These factors did not alter NE responsiveness of VSM from controls. From these results, it is suggested that the sodium-potassium pump regulates vascular α-adrenergic responsiveness and that the response to NE is affected more in the DOCA/NaCl rat by alterations in the sodium-potassium pump. This would be expected since the activity of the pump is increased in the DOCA/NaCl hypertensive rat. These results can be extrapolated to the whole animal in that if there is a circulating inhibitor of the sodium-potassium pump then, based upon these results, an increase in VSM responsiveness to NE would be expected. This could lead to an increase in peripheral vascular resistance and eventually hypertension. However, the actual time course of alterations in pump activity and the occurrence of a ouabain-like factor have not been investigated.

The following are additional conclusions regarding the experiments in this dissertation:

1. Maximum active tension developed at a preload force of 1.0 gram for VSM from both control and DOCA/NaCl hypertensive rats. The lack of a difference in this value between the two groups suggests minimal structural alterations in the VSM of DOCA/NaCl hypertensive rats. Ring weight was slightly greater in the DOCA/NaCl group.

2. Incubation of VSM from both groups with the α-adrenergic antagonist, phentolamine, increased the relaxation response to ISO only slightly, but significantly attenuated the contractile component of this β-adrenergic agonist. It is suggested that the contraction induced by ISO be used as an indicator of α-adrenergic responsiveness since the response was greater in the DOCA/NaCl hypertensive rat. In addition, it is suggested that phentolamine not be used since it is known to have nonspecific effects on contraction and relaxation of VSM.

3. Phentolamine also decreased the maximal contractility of VSM from DOCA/NaCl hypertensive rats in response to KCl. However, no effect on either the sensitivity to KCl in the DOCA/NaCl rat or on rings of femoral artery from controls was observed. This suggests

that the increase in contractility in VSM from DOCA/NaCl hypertensive rats in response to high KCl concentrations may be due to a release of NE from nerve terminals. It must be suggested again, however, that phentolamine may have nonspecific effects which decrease the response to KCl.

4. Although maximal relaxation in response to ISO was not affected by low calcium Krebs, the sensitivity to ISO was increased in both controls and DOCA/NaCl rats. It may be suggested that a defect in membrane handling of calcium by the VSM contributes a minor role to the decreased β-adrenergic responsiveness in the DOCA/NaCl treated rat. This is supported by the fact that a high calcium (7.5 mM) Krebs solution decreases the response to ISO.

5. The response of VSM from controls and DOCA/NaCl hypertensive rats to KCl and NE were slightly decreased in the high calcium media. This author suggests that the decrease in response may be due to a membrane stabilizing effect of calcium which decreases the ability of calcium itself to permeate the VSM cell membrane. The results of the calcium relaxation experiment support this. Femoral smooth muscle precontracted with NE relaxed in response to addition of 7.25 mM $CaCl_2$.

6. Potassium-relaxation of VSM from DOCA/NaCl hypertensive rats was enhanced over controls. Maximal relaxation occurred at approximately 2.0 mM potassium in DOCA/NaCl rats. Controls, however, did not respond

maximally until approximately 4.0 mM potassium. It appears that the sodium-potassium pump is maximally active at around 4.0 to 6.0 mM potassium in controls, or in the normal physiological range of potassium concentration. On the other hand, the sodium-potassium pump in VSM from DOCA/NaCl rats is "physiologically active" at potassium concentrations of around 2.0 mM. This suggests a large reserve in terms of required pump activity in the DOCA/NaCl rat which is most likely a compensatory phenomenon.

7. Ouabain-sensitive rubidium-86 uptake was significantly greater in the DOCA/NaCl rat, however, ouabain-insensitive uptake was similar to controls. If the increase in pump activity is a compensatory phenomenon it is not due to an increase in passive ion movements. Therefore, it is suggested that the increase in pump activity is a result of a compensation in response to a ouabain-like pump inhibitor.

8. The rate of rubidium-86 uptake was fairly constant in controls for each time interval, however, in VSM from DOCA/NaCl hypertensive rats the rate decreased progressively with increasing incubation time. This observation further reflects the increased pump activity and suggests that maximum rate of uptake occurs sooner in the DOCA/NaCl rat.

9. Calcium relaxation was significantly greater in DOCA/NaCl hypertensive rats. In addition the response was dependent upon the activity of the sodium-potassium pump.

That is, with decreasing pump activity (low potassium, ouabain) the response to calcium was decreased and with increasing pump activity (10 mM potassium) the response to calcium was increased. These results are in agreement with the potassium-relaxation and rubidium-86 uptake experiments in that sodium-potassium pump activity is increased in the DOCA/NaCl hypertension. In addition, it is suggested by this author that calcium relaxation may be used as another index of pump activity since the response was blocked by ouabain and was potassium concentration-dependent.

10. The response of VSM to NE was investigated at various levels of sodium-potassium pump activity. One way to decrease pump activity is by decreasing the temperature in the smooth muscle bath. In this dissertation, pump activity was decreased approximately 50 percent by decreasing the temperature from 37° C to 27° C. In order to determine that alterations in VSM responsiveness to NE are not a result of the temperature change, KCl dose-response curves were generated. It was observed that the response to KCl was not affected in either group when compared to responses in normal conditions. Therefore, it is suggested that alterations in the response to NE are a result of decreased pump activity and not due to a change in the basic contractile mechanisms.

11. Relaxation in response to ISO in conditions of altered pump activity were investigated following contraction

with serotonin. KCl was not used since alterations in pump activity were achieved by altering extracellular potassium. ISO-induced relaxation in normal potassium following contraction with serotonin was similar to responses in VSM precontracted with KCl for both groups. The responses of VSM from DOCA/NaCl rats in altered potassium, however, were quite complex, since decreasing pump activity actually increased ISO-induced relaxation. These results were not expected since it was hypothesized, and has been suggested by others, that ISO-induced relaxation is mediated in part by the sodium-potassium pump. Therefore, with decreasing pump activity the response to ISO would be expected to decrease. It is suggested that a complex interaction between ISO, serotonin, and pump activity occurs which alters the response to ISO in the DOCA/NaCl rat. It can also be suggested that the alteration involves a defect in the response of VSM to any one or a combination of these agents since no appreciable affect was seen in control animals to these conditions.

12. Serum concentrations of T_3 and T_4 were determined in control and DOCA/NaCl hypertensive rats. A decrease in T_4 was seen in the DOCA/NaCl rat when compared to controls while T_3 was slightly elevated. In addition, thyroid weight was significantly increased in the DOCA/NaCl treated rat. This suggests an alteration in the thyroid state of the DOCA/NaCl animal. The role of the

thyroid in the development of DOCA/NaCl hypertension remains to be elucidated. Since thyroid hormone is known to affect the sodium-potassium pump, and the pump is altered in the DOCA/NaCl rat, it was of interest to this author to determine serum thyroid hormone concentrations in the DOCA/NaCl hypertensive rat. Although no definitive statement or correlation can be made about the vascular sodium-potassium pump and thyroid hormone levels, it can be suggested that the two may be interrelated and further studies are warranted.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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